Spring 2021

Insulin: Roles and Functions in Biochemistry and U.S. Healthcare

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There is a long list of drugs that treat conditions affecting a large population of the U.S. today. Genetic predisposition, individual lifestyle, and environmental factors all influence many of the most prominent diseases in our population, and the development of drugs as a treatment strategy has brought about both burdens and benefits for the patient. One example of one of these drugs is insulin, with 30% of Diabetes patients using insulin.[1] Today, over 8.3 million people are prescribed insulin, and that number is expected to grow substantially over time.[1] This massive market is controlled by a small number of manufacturers, causing high cost of insulin and low accessibility for patients. While a higher percentage of the U.S. population has Type 2 Diabetes than Type 1, I will be focusing on Type 1 because of its more limited range of treatment options.

Type 1 Diabetes occurs in over 30 million Americans.[1] It is caused by an autoimmune process in which CD8 positive cytotoxic T cells recognize beta-cell autoantigens by recognizing MHC class I peptide complexes. This produces a chronic inflammatory response to eliminate insulin-producing beta cells, causing insulin deficiency and hyperglycemia.[2] In previous studies, glutamic acid decarboxylase (GAD) and ICA 512 protein tyrosine phosphatase (in 60-85% of patients) have been found to help in elimination of insulin.[2]

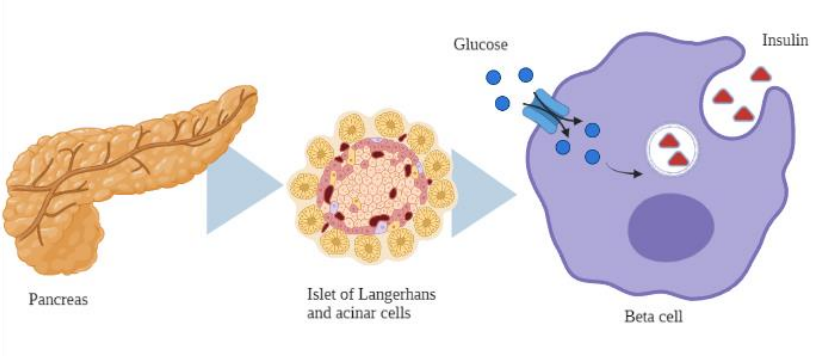


Figure 1. Insulin leaves β-cells of pancreatic Isles of Langerhans when stimulated. Pipeline includes the pancreas, an Islet of Langerhans surrounded by acinar cells, and β-cell that is stimulated to produce and release insulin. Image created using BioRender.

Insulin is naturally produced by the body, secreted by pancreatic beta-cell in the Islets of Langerhans as a 51-residue protein.[3] This release

is pictured in **Figure 1**, showing the release of insulin from pancreatic β-cells. The initial protein, proinsulin, has an A and B chain connected by disulfide bonds, as shown in the **Figure 2**, which is post-translationally modified to form mature insulin.[3]

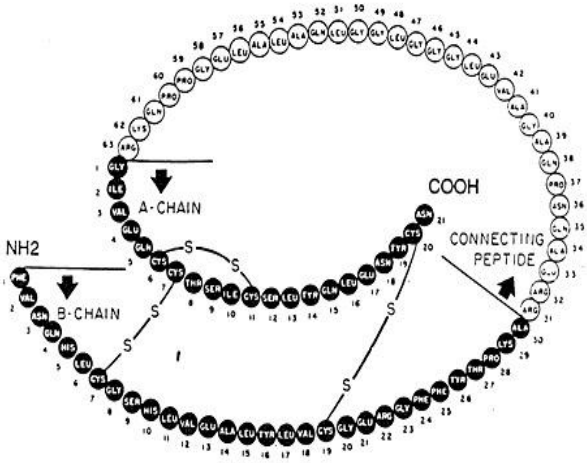


Figure 2. Animation of insulin molecule.[3]

Insulin contains three alpha helices: A1-A8, A12-A18, and B9-B19, containing one intra and two interchain disulfide bonds[4] (**Figure 3**).

Insulin works by binding to the insulin receptor (IR), a homodimer with two alpha subunits, each at 135 kDa; two beta subunits, each at 95 kDa; and two minor glycoprotein (gamma) subunits, each at 210 kDa.[5] The alpha subunit works to bind insulin, and the beta subunit consists of a tyrosine kinase that phosphorylates itself at a phosphotyrosine, phosphoserine, and phosphothreonine (**Figure 4**). The gamma subunit is the transmembrane portion, extending through the plasma membrane to connect to the inside of the cell.

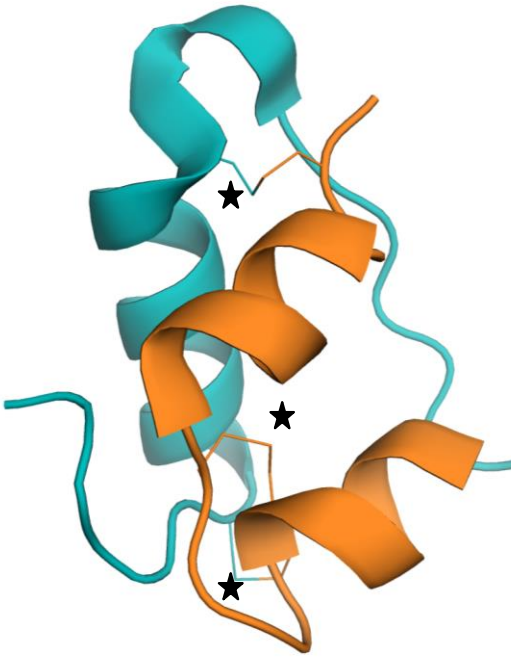


Figure 3. Structure of insulin molecule. Alpha and beta chains are colored orange and teal, respectively, with intra and inter-disulfide bonds labeled with stars.

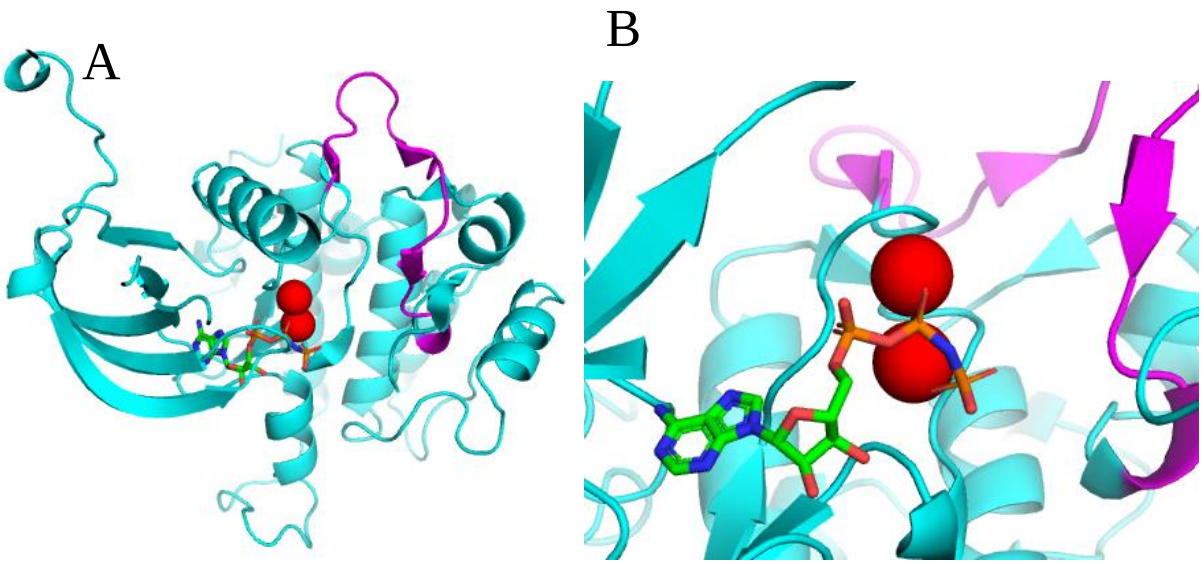


Figure 4. A) Overview of tyrosine kinase subunit of insulin receptor protein. Tyrosine kinase is colored cyan, the swinging loop region is colored magenta, the two Mg^{2+} coenzymes are colored red, and multicolored is an ATP analogue, with carbon green, nitrogen blue, oxygen red, and sulfur orange.

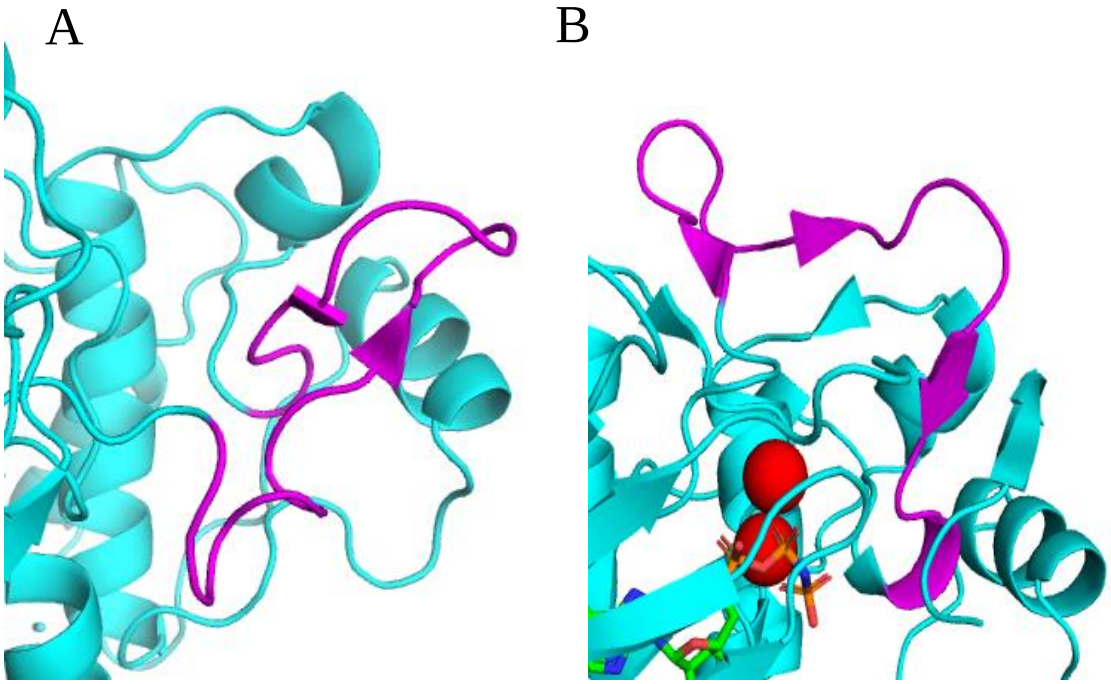


Figure 5. Tyrosine kinase undergoes transitional change upon ATP binding. A) Inactivated tyrosine kinase region of insulin receptor protein. Highlighted in magenta is part of the loop that swings during activation, to allow entry into the active site. B) Activated tyrosine kinase, highlighting the loop swing.

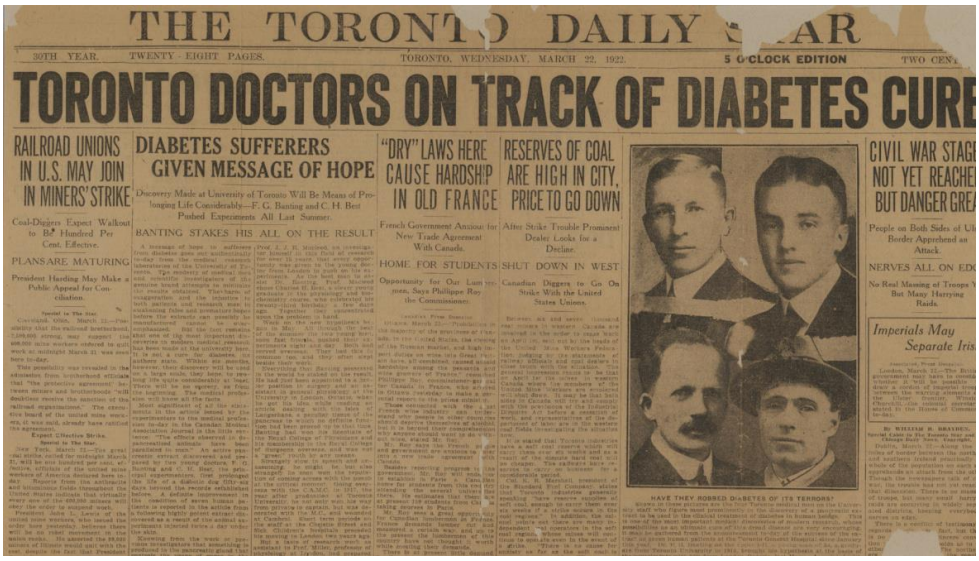
When insulin binds to one of the receptor's alpha subunit, it causes a shape change that is propagated into the intracellular region and activates the tyrosine kinases.[6] The transition from the inactive to the active state is shown in **Figure 5**. The active site binds to ATP, which it uses to phophorylate its targets. The transition between the inactive and active state is characterized by several tyrosines being phosphorylated, causing a loop portion to swing out of the active site and allow ATP and magnesium entry for enzymatic process.[6]

All of this information that's known about the insulin protein and insulin receptor is helpful to researchers to maximize efficiency of Diabetes drugs today, but this information has not been known nearly as long as Diabetes has been around. Treatments to Type 1 Diabetes, prior to the use of insulin as a drug, were trying and physically exhausting. Developed in the early 20th century, starvation therapy was a way to keep sugar below tolerance levels, measured routinely in urine. This would hopefully prevent organ failure, but it meant that a patient had to restrict themselves to 800 calories per day.[7] This caused weakness and low resistance to infections, as well as a generally decreased quality of life. Without treatment, diabetes would start with weight loss, listlessness, hunger, and thirst, as ketones clog organs and the kidneys begin to fail. Eventually, coma and then death would ensue. Treatments were becoming more and more important as the 20th century brought cleaner water and better sewage. As child mortality rates declines, the rates of Type 1 Diabetes increased with the growing population. Type 2 also increased, as being larger was a sign of wealth and prosperity. A better treatment was imperative.

However, it wasn't for lack of trying that the treatment development was slow. Since 1869, when Paul Langerhans identified insulin-secreting pancreatic cells that floated in clusters of acinar cells—these clusters called islets of Langerhans—there was work on cats, dogs, guinea

pigs, and rabbits to find pancreatic extract for treating patients.[7] One group in 1912 thought that bacterium in milk would cleanse the alimentary canal from excess sugar. Patients were, of course, told to drink more milk, not knowing of the sugar contained in what they were drinking. In 1915, J.D. Rockefeller founded a medical research institute, let by Israel Kleiner, to work on pancreatic extracts. Kleiner's method was to grind up dog pancreases, put them in solution with salt, and inject them into the now-diabetic dogs. He found 50% decline in blood sugar levels, but the negative side effects prevented further investigation.[7]

The world-changing science was in 1914, when a group of scientists at University of Toronto banded together to find a way to extract insulin (**Figure 6**). The team was led by J.J.R. Macleod and included F.G. Banting, C.H. Best, J.B. Collin, J.Hepburn, J.K. Latchford, and E. Clark Noble. They used rabbits and developed manufactured pancreatic extract, first calling it "isletin." At first in an oral form—given to Banting's friend Dr. Joseph Gilchrist—with no beneficial results, it was later successfully given as an injection to Leonard Thompson, a 14-year old patient of Toronto General Hospital.[8] The Toronto group, on January 25, 1922, settled an agreement with Connaught Anti-Toxin Laboratories for the manufacturing of this extract, but the production failed by March. Collin and



THE TORONTO DAILY STAR

20TH YEAR. TWENTY-EIGHT PAGES. TORONTO, WEDNESDAY, MARCH 22, 1922. 5 O'CLOCK EDITION TWO CENTS

TORONTO DOCTORS ON TRACK OF DIABETES CURE

RAILROAD UNIONS IN U.S. MAY JOIN IN MINERS' STRIKE

Coal-Diggers Expect Walkout to Be Hundred Per Cent. Effective.

PLANS ARE MATURING

President Harding May Make a Public Appeal for Conciliation.

DIABETES SUFFERERS GIVEN MESSAGE OF HOPE

Discovery Made at University of Toronto Will Be Means of Prolonging Life Considerably—F. G. Banting and C. H. Best Probed Experiments All Last Summer.

BANTING STAKES HIS ALL ON THE RESULT

"DRY" LAWS HERE CAUSE HARDSHIP IN OLD FRANCE

French Government Anxious for New Trade Agreement With Canada.

HOME FOR STUDENTS

Opportunity for Our Lumbermen, Says Philippe Roy the Commissioner.

RESERVES OF COAL ARE HIGH IN CITY, PRICE TO GO DOWN

After Strike Trouble Prominent Dealer Looks for a Decline.

SHUT DOWN IN WEST

Canadian Diggers to Go On Strike With the United States Unions.

HAVE THEY ROBBED DIABETES OF ITS TERRORS?

CIVIL WAR STAGE NOT YET REACHED BUT DANGER GREA

People on Both Sides of Ul Border Apprehend an Attack.

NERVES ALL ON ED

No Real Massing of Troops But Many Hurrying Raids.

Imperials May Separate Iris

Figure 6. Cover of Toronto Daily Star, published on March 22, 1922, showing the four doctors leading the insulin treatment research.

Best succeeded in regaining the formula in May. By May 3rd, the extract was successfully extracted from cow pancreas and was first referred to as insulin by Macleod. Elizabeth Hughes Gossett, daughter of U.S. statesman Charles Evans Hughes, was the famous recipient of the first insulin treatment for Type 1 diabetes, receiving over 42,000 shots from age 12 until her death.[7]

Banting refused to put the patent for insulin in his name, believing it would be unethical for a doctor to benefit from the discovery of a life-saving treatment; Collin and Best respected this decision and agreed to sell the patent for insulin for $1 to the University of Toronto. The intention was to provide the quickest availability to the public and allow anyone to prepare the extract with no profitable monopoly to develop.[9] While this was meant to prevent monopolizing on the drug production and distribution, it did little in the end for the benefits of patients. The University formed the Diabetic Clinic in Toronto General Hospital in June of 1922 and established the Insulin Committee in August to control licensing, patenting, and trademarking of the new drug.[8] The teamed with Eli Lilly to allow it to take out any U.S. patents "for manufacturing improvements, but the university would receive the patent right for the rest of the world, and they let other companies around the world receive the patent from the University.[9] By May of 1923, insulin was made commercially available in Great Britain, and by June, the initial agreement with Eli Lilly ended, allowing other pharmaceutical companies to apply for licenses for distribution. By October of 1923, insulin was made commercially available in the U.S. and Canada. The Nobel Prize in Physiology or Medicine was given to Banting and Macleod that same month, for their discovery of insulin.[10]

Over the next three years, production was massive. The world began hearing of the new drug to treat Diabetes, and what rose was a new hope in patients for a normal way of life. By 1926, insulin was patented or trademarked in 44 countries world-wide. Through the next 100

years, continual work was done to maximize its productivity in the body. At Novo Nordisk, one the of three companies controlling the insulin market today, Hans Christian Hagedorn added protamine to the drug to alter its absorption and prolong its duration of action. This was not able to mix with fast-acting insulin, so he added zinc to the drug and formed neutral protamine Hagedorn (NPH). This allowed a single daily injection for many patients and was patented in 1946.[9] Slow-acting insulins were introduced in the 1950s to extend patents into the 70s, then introduced monocomponent insulins and single-peak insulins—by Novo Nordisk and Eli Lilly, respectively—which extended patents into the 80s.

Genentech, a small start-up biotechnology firm with no income and limited resources, was the first to synthesize and patent human insulin the 1970s.[11] They incorporated the gene for insulin into *E. coli*—a process called DNA recombination—to harvest the protein from bacterial fermentation. Human insulin was the first protein to be FDA-approved for treatment using this method (**Figure 7**).[12] This was only accomplished only a couple years before DNA recombination and purification was first done and published. With a royalty agreement with Eli Lilly, human insulin hit the market as Humulin R in 1982[9] and instigated a surge in investment in biotechnology. And it worked better for patients.

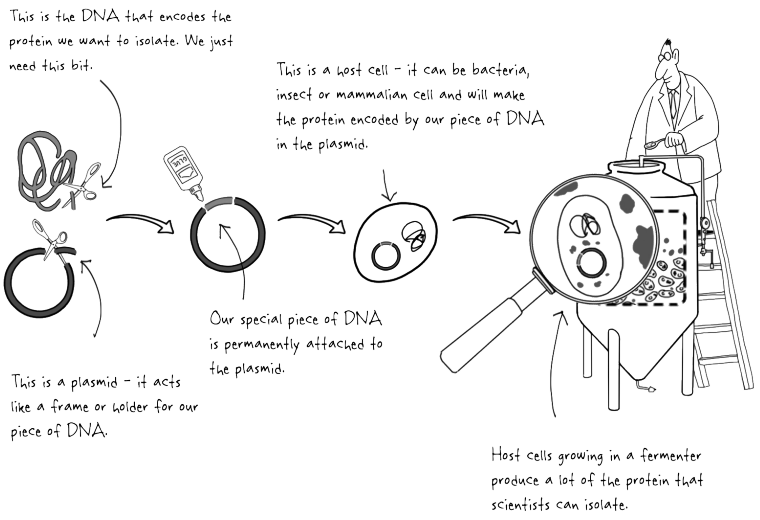


Figure 7. Illustration of the recombination process to produce isolated human insulin.[12]

Only six years later, Nordisk brought its first human insulin to market, using biochemical methods to convert

bovine insulin to human insulin.[9] Not long after, Sanofi did the same, and human insulin would determine the three companies to own the entire insulin market for the following forty years.

Today, insulin treatment is much more than a single-type injection that is the same for every patient. In 1977, human insulin was synthetically manufactured from recombinant DNA and replaced beef and pork insulin.[13] Today, insulin analogs are replacing human insulin because they are less likely to clump together in high concentrations under the skin, causing slow and unpredictable absorption from subcutaneous tissue the way human insulin does. Analogs have a more predictable duration of action, and work quicker, last longer, and have a less peak-like effect. As of 2010, only 15% of the insulin market uses human insulin, while 92% use analog insulin.[14]

There are three types of insulin. Fast-acting insulin, as the name suggests, is absorbed quickly from subcutaneous tissue into the bloodstream and is used during meals to regulate blood sugar levels.[13] These include rapid-acting insulin analogs—such as Insulin Aspart, Lyspro, and Glulisine—with an onset time of 5-15 minutes, a peak effect of 1-2 hours, and an overall duration of 4-6 hours. There is also regular human insulin, with an onset time of 30 minutes-1 hour, a peak effect of 2-4 hours, and a duration of 6-8 hours. Another type is intermediate-acting insulin, which absorbs slower and lasts longer. This is used overnight, while fasting, and between meals. It includes NPH human insulin, with an onset of 1-2 hours, a peak effect of 4-6 hours, and a duration over 12 hours; and premixed insulin, a mixture of NPH and regular human insulin of rapid-acting analog. This gives a combination of short and intermediate-acting insulins. The third type is long-acting insulin, which is absorbed slowly, has minimal peak effect, and a stable plateau over most of the day. This is used overnight, while fasting, and between meals, and it mainly consists of long-acting insulin analogs—such as Insulin Glargine and Detemir—with an

onset of 1.5-2 hours, when the effect plateaus then holds a flat duration of action of 12-24 hours.[13] **Figure 8** visualizes these effects.

Most patients with Type 1 Diabetes must take basal insulin throughout the day, which controls insulin levels between meals, as well as mealtime insulin to control those levels during meals, when glucose intake spikes.[14] A typical day consists of one or two injections of long-acting, and rapid-acting for every meal. Type 2 patients may only need long-acting insulin injections. Most patients still use syringes as the delivery method. While insulin pens combine the vial and syringe, being safer and more convenient, they are used less because of higher cost. CSII devices—Continuous subcutaneous insulin infusion devices—are battery-powered, computerized, and deliver short-acting insulin under the skin continuously throughout the day via an abdominal catheter. These are also expensive but can last years and are commonly used. Another delivery method is infusion, using a direct IV, but this is reserved for patients when in a hospital. A new method is inhaled insulin, which is rapid-acting and meant to be used before meals, in combination with long-acting insulin.

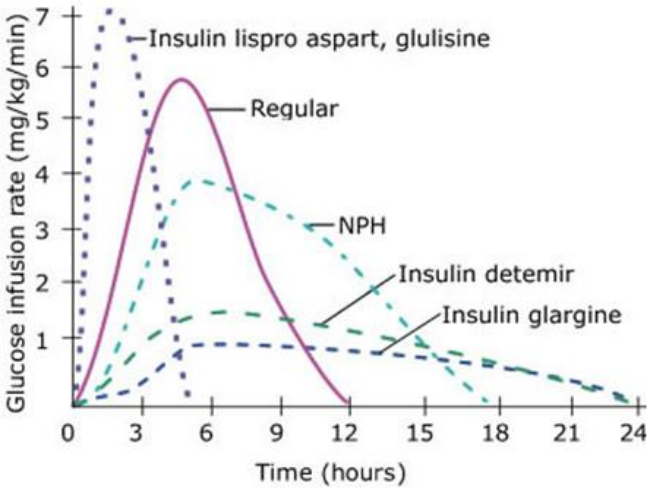


Figure 8. Activity profiles of different types of insulin on the market today.[13]

There are other medications to treat insulin today that are not insulin, called oral antidiabetic drugs (OADs). One is Metformin, a treatment for Type 2 Diabetes that most commonly works in conjunction with insulin. It has been used since the 1950s, only briefly taken

off the market due to concerns of lactic acidosis, and acts by blocking a mitochondrial redox shuttle to inhibit gluconeogenesis in the liver.[15] Another class of drugs are glucagon-like peptide 1 (GLP-1) receptors, which also work in Type 2 Diabetes patients to increase insulin secretion in response to oral and intravenous glucose.[16] Another common medication includes sodium glucose co-transporter 2 (SGLT-2) inhibitors. These work in combination with insulin for Type 1 and Type 2 Diabetes. SGLT-2 inhibitors block the reabsorption of glucose by the kidney and reducing blood glucose levels as glucose is excreted from the body.[17] While these classes of drugs provide promising progress toward helping those with Diabetes, they are commonly only given to Type 2 patients and almost always still work in conjunction with insulin. Making OADs more accessible to Type 2 users is a worthwhile endeavor, but the significant problem still remains of providing all DM affordable access to the still most-widely used medications, insulin.

Over 8.3 million people in the U.S. require insulin. And the worldwide market is expected to increase by at least 20% by 2030.[14] To begin with the problems of the insulin market, there is limited competition for three major reasons. Insulin products are not interchangeable, there are currently only three insulin manufacturers in the U.S. market, and there have been no regulations to allow biosimilar products to form a generic insulin.

The cost of insulin today is outrageous. From 2002 to 2016, the price of insulin has increased by 218%.[18] The last four years of that period, the increase was 450% above general inflation. Estimated cost of spending, *per patient*, has increased from $231.48 in 2002 to $736.09 in 2013.[19] And the average price of insulin has increased from $4.34 to $12.92 per milliliter from 2002 to 2013. These are partially due to continually newer and “better” insulin products coming to market, raising the cost and patents, but these increasing costs are discouraging patients from

wanting to switch to newer insulin when they do work better. Many patients are afraid of not being able to pay their medical bills or of losing their health insurance altogether.

While the high cost of insulin is not new or that surprising, it affects millions of Americans each year. In a 2019 Yale study, 25.5% of insulin users reported cost-related insulin underuse.[20] This is both dangerous and unacceptable, and highlights "an urgent need to address affordability of insulin."

Because of non-interchangeable insulin products, there is little competition in the insulin market. There are large differences in insulin types, so patients cannot easily change them.[14] With this, there is little autonomy in patient decisions on what form of insulin is best for them, and changing forms is left for the provider to decide.

The three insulin manufacturers are Eli Lilly, Novo Nordisk, and Sanofi, representing over 90% of the global insulin market.[14] While each of these companies have multiple types, Novo Nordisk have a monopoly on ultra-long acting insulin. This is because of patents. Patents last 20 years, and companies can receive a new patent every time they improve the way to produce or administer the drug, or with changes in duration or onset time. For insulin specifically, this can be for using new, non-active ingredients, changing the manufacturing processes, or developments in administration devices. Patents have followed the development of insulin through time, starting with animal insulin in 1923, the first human insulin in 1982, and new analog patents from 1996-2005.[14] When a new product comes to market, older products are less prescribed and often discontinued from the manufacturer. This prevents patients from having access to off-patent insulins, which would potentially be cheaper. If one looks at the minor changes in insulin from 1996 to 2005, the price increase of the newly-patented insulin is often not balanced by equally-improved effects. For example, analogue insulin has shown improved

effects compared to human insulin, as discussed earlier, but the estimated expenditure per patient in 2013 was $228.20 for human insulin and $507.89 for analog, making it significantly less affordable.[19]

To complicate things, insulin has been considered a drug rather than a biological drug or biologic. This designation does not allow generic versions to be made when the patent expires. As of 2009, however, the FDA created a legal path for biosimilars to reach the market, called the Biologics Price Competition and Innovation Act.[21] This changed the designation from a drug to a biological product. Other companies can now create bio*similar* product—where they would previously be called generics—where the molecular and biological structure doesn't show any clinically meaningful difference from the biologic. This provision took effect on March 23, 2020, and is predicted to lower the cost of insulin as other companies produce these biosimilars.

This transition of insulin's designation should not be discounted. Generics are an enormous market in the drug industry. Over 80% of prescriptions are for generic drugs; this saves health care systems *billions* of dollars each year and are critical for payers and patients.[9] And yet, there is some skepticism in the savior mentality of producing generics to fix the insulin price. With this Act, insulin companies are still allowed the usual patents of 20 years when they introduce "improvements," and with such a large molecule, there is a large number of possible incremental changes. This is the process has been continuing since insulin first came to market and is referred to as "evergreening." Developing a biosimilar is incredibly challenging, as biologics are huge molecules compared to drugs. Creating biosimilars take about twenty-two times the cost of creating a small-chemical molecule drug, and this expense will slow competitors and possibly prevent any price drops.[14] It is incredibly difficult to prove that two molecules are structurally identical, which is necessary for a generic. There is also a more

extensive approval process that will slow progress and add expenses. This is the case in Europe: recently, they have introduced generic insulin, but the price reductions were 20-40% instead of the usual 80% for introduction of a generic.[9]

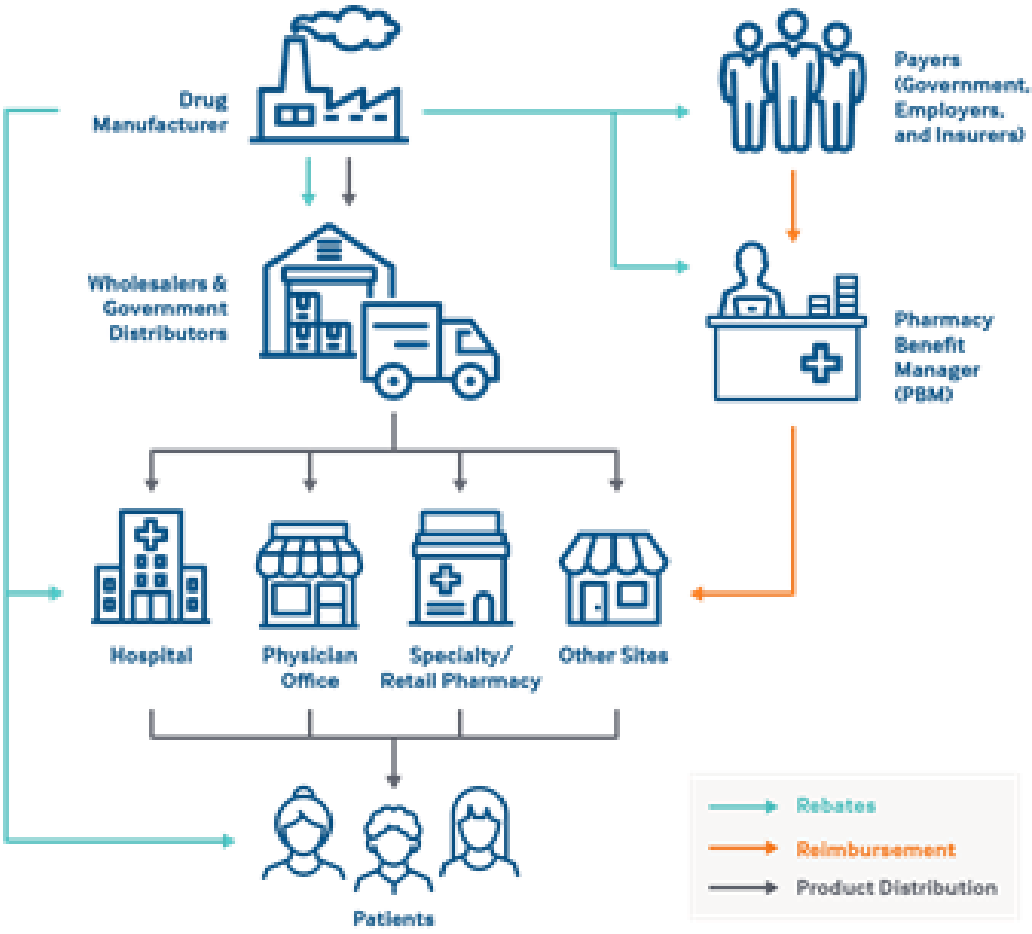


Figure 9. Pharmaceutical supply chain of medicine distribution.[1]

A study done by The Commonwealth Fund suggests a different tactic in increasing affordability: Increase both the access to insurance coverage and the generosity of that coverage for insulin patients.[1] Reporting that as many as ¼ of insulin patients report rationing insulin to save money, policies need to be implemented to expand Medicaid and limit out-of-pocket spending for those with private insurance. Commonwealth goes into detail about the complicated process of insulin reaching the user, shown in **Figure 9**. Manufacturers give rebates to PBMs, or

Pharmacy Benefit Managers, and collect the entire net price of insulin minus those rebates, fees paid to wholesalers, and discounts paid to pharmacies. Each of these payments are kept at a specific percentage of the overall list price, giving the manufacturers incentive to keep the price high, benefiting everyone but the user.

Ultimately, whether reducing list prices, limiting deductibles, expanding Medicaid, or improving health insurance is the solution depends on whether the user has insurance or not. While those with Medicaid pay nothing out of pocket, uninsured users pay list prices, unless they're part of manufacturer programs or free clinics, which are rare. Thus, expanding Medicaid could be an option to help many low-income users, who often try to use older insulin formulations because of lower prices, which are associated with higher risks of hypoglycemic episodes, nocturnal hypoglycemia, and increased within-subject variability of blood glucose.[1] For uninsured prescriptions (those without Medicaid), 47% were paid 100% out of pocket and only 21% had 0% out-of-pocket cost. Additionally, 71% of uninsured prescriptions were more than $100 out of pocket.[1] Patients are often uninsured because of low socioeconomic status, and these high prescription costs add significant financial stress.

Those with are privately insured and under 65 pay varying out-of-pocket costs, consisting of copayments or an insurer-established price, up to a deductible, plus a coinsurance amount up to an out-of-pocket max. Lowering these deductibles and maximums is another strategy, and progress has been made here: In Washington State, the Senate bill 6087 issued a state-wide cap of $100 per month that began on January 1, 2021.[22] This cap has been introduced to several western states in the last three years.

The Affordable Care Act (ACA), enacted in 2010 under the Obama administration, required all states to expand Medicaid eligibility to adults with incomes below 138% of the

federal poverty level.[23] The US Supreme Court ruled that expansion could be voluntary for each state. In 2014, twenty-nine states expanded Medicaid eligibility, and the effects were observed. Many were general improvements, such as easier access to primary care, increased health care

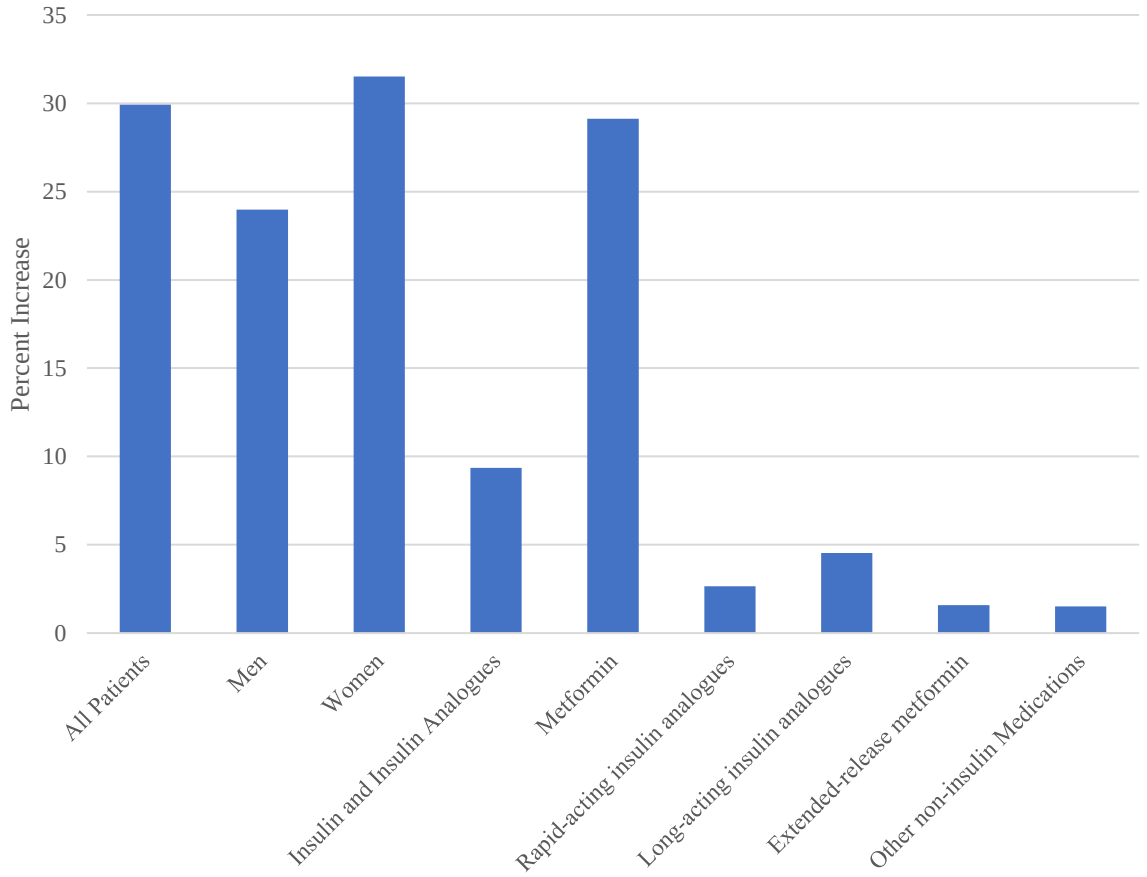


Figure 10. Percent increase in Diabetes medications over expanded Medicaid eligibility. Changes recorded as averages over 2014 and 2015, as 29 states expanded in both years. Other non-insulin Medications include DPP-4 inhibitors, GLP-1 agonists, and SGLT-2 inhibitors.

use, and increase in the number of Medicaid prescriptions. A study done by Myerson, et al. aimed to measure the change in different types of Medicaid diabetes prescriptions when the eligibility expanded, hoping to see whether the expansion helped lower-income patients with diabetes progress to using the newer and better medications.[23] As shown in **Figure 10**, the change in Diabetes medications showed significant increase over the period of eligibility

expansion from 2014 to 2015. Insulin, insulin analogues constituted about a third of the increase at 9%, indicating easier access. The last four columns track newer medications and show that as much as a third of the increase was likely to newer medications for patients who previously lacked insurance and could not pay the high out-of-pocket costs.[23] Additionally, the increase in prescription fills was significantly higher as patients reached sixty-five, following the trend of Diabetes prevalence increasing with age. The exception was an 82% lower increase for people 65-69 than for people ages 60-64, which was expected, as the older age group is also eligible for Medicare, which will be used first. Overall, the study revealed an average of thirty new Medicaid prescriptions per 1,000 people in states that expanded eligibility.[23] Prescriptions for insulin types cumulatively reached nearly a 40% increase after expansions, revealing the greater access when the expensive full costs of insulin are covered by Medicaid. Myerson asserts that "gaining Medicaid insurance would have significantly reduced out-of-pocket spending for insulin for previously uninsured patients, thereby facilitating uptake of the medication." Expanding Medicaid not only saved people money or encouraged use of insulin, but encouraged use of *newer* insulin for lower-income patients.

Thus, there are several ways to increase insulin accessibility. Allowing generic production, regulation of list prices and deductibles for those with insurance, and especially expanding Medicaid for those without insurance are all promising ways to cumulatively increase access and affordability of insulin and insulin analogues. The focus needs to be on accessibility on the newer insulin treatments for those with lower incomes. As insulin remains to be imperative for treatment of Diabetes for all patients, we need to address insulin accessibility in policy initiatives to work toward expansion.

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