Design for Biosecurity

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Lecture 34

Insulin Chemistry

Let's begin today's class. As we discussed in the previous session, today we will delve into the chemistry of insulin and its physiology. As I mentioned earlier, insulin is a peptide. If you examine its empirical formula, insulin consists of 254 carbon atoms, 377 hydrogen atoms, 65 nitrogen atoms, 75 oxygen atoms, and 6 sulfur atoms. It has a molecular weight of approximately 5734. Insulin is constructed from 51 amino acid residues, making it one of the smallest proteins in the human body.

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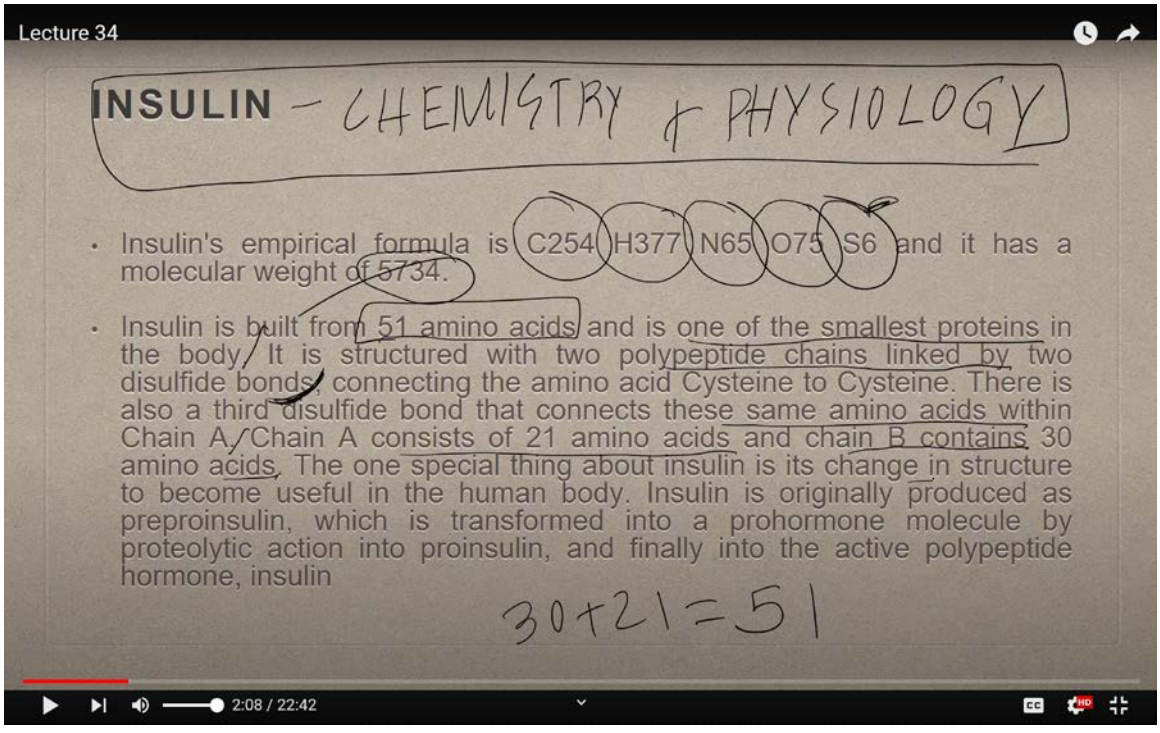

Structurally, insulin is fascinating. And while we refer to it as one of the smallest proteins, it was actually the work of James Bertram Collip that enabled us to understand how to

isolate insulin from a mixture of pancreatic secretions. Insulin consists of two polypeptide chains that are linked by two disulfide bonds. These disulfide bonds are formed by sulfur atoms, which play a crucial role in connecting the amino acid cysteine to another cysteine residue. Additionally, there is a third disulfide bond within Chain A that links the same amino acids. Chain A is composed of 21 amino acids, while Chain B contains 30 amino acids, bringing the total to 51 amino acids.

One particularly remarkable aspect of insulin is how its structure changes to become biologically active in the human body. Initially, insulin is produced as a precursor molecule called pre-pro-insulin. Through proteolytic processes, pre-pro-insulin is first converted into pro-insulin and then finally into the active polypeptide hormone insulin.

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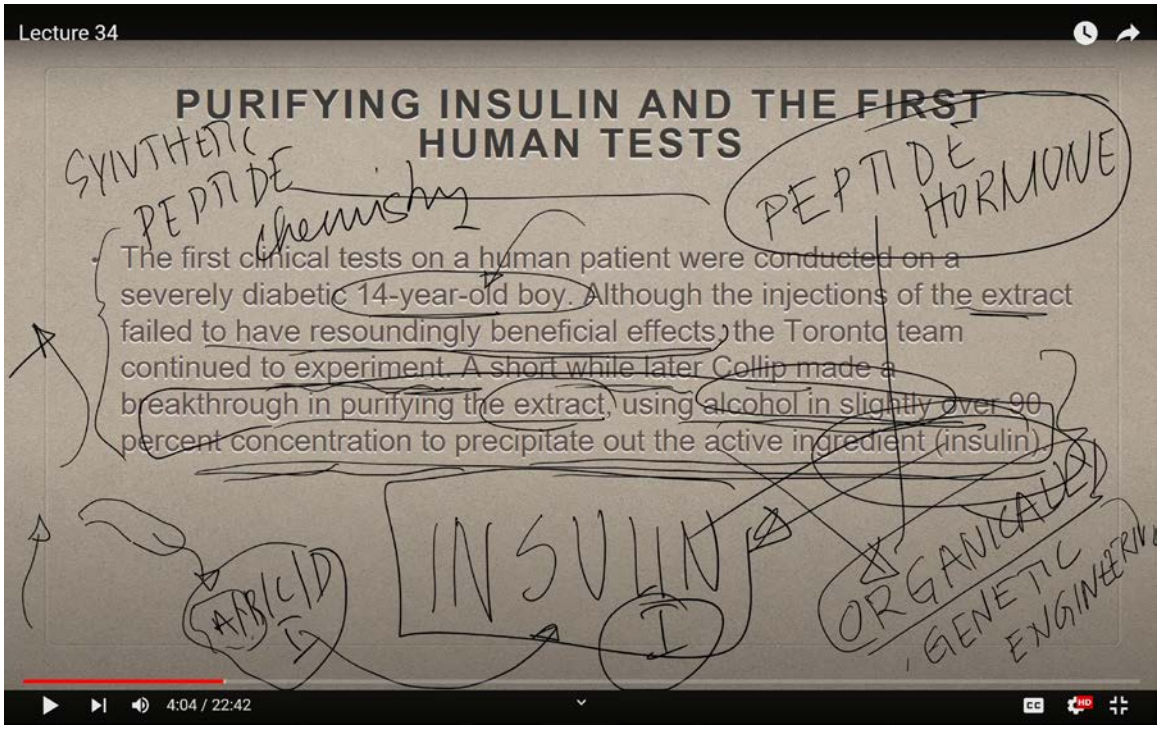

The key takeaway here is that insulin is not produced in its active form; it undergoes multiple stages of processing and has protective elements before it becomes functionally active. This means that whenever we extract insulin, we must ensure that it is in its active form. This was crucial in the early stages of insulin research. For instance, during some

early experiments, the first clinical trial on a human patient was performed on a severely diabetic 14-year-old boy. Although the initial injection of the insulin extract did not have a profoundly positive effect, the research team in Toronto persisted. Shortly thereafter, Collip made a significant breakthrough in purifying the insulin extract by using an alcohol concentration of slightly over 90% to precipitate out the active insulin ingredient.

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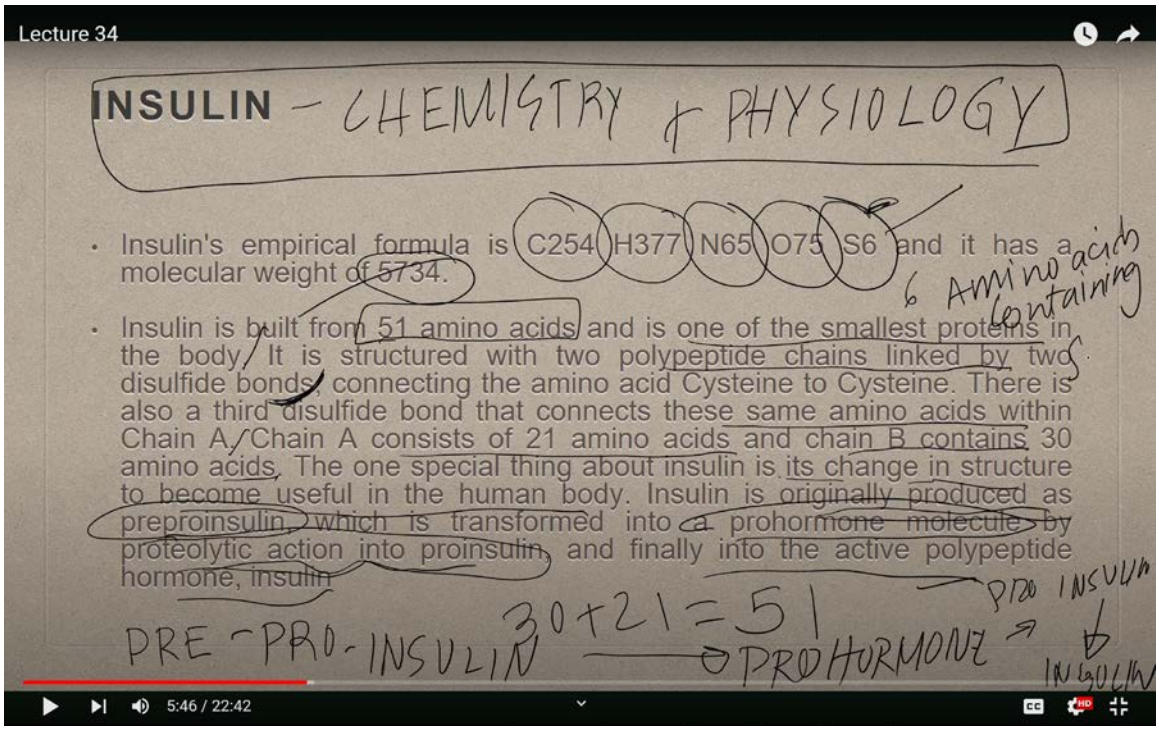

This breakthrough allowed insulin to be successfully used as a treatment, paving the way for its vital role in managing diabetes today.

This word insulin itself is the active ingredient of insulin. Now, it's important to understand that in some experiments, insulin might not be in its fully active form. Instead, it could be in its precursor forms, such as pre-pro-insulin or pro-insulin. So, when an experiment seems ineffective, you must pause and consider multiple factors before drawing conclusions. It’s essential to remember that working with biological extracts, taking a fluid from one organism and introducing it into another, presents significant challenges, and replicating the exact response outside the body can be incredibly complex.

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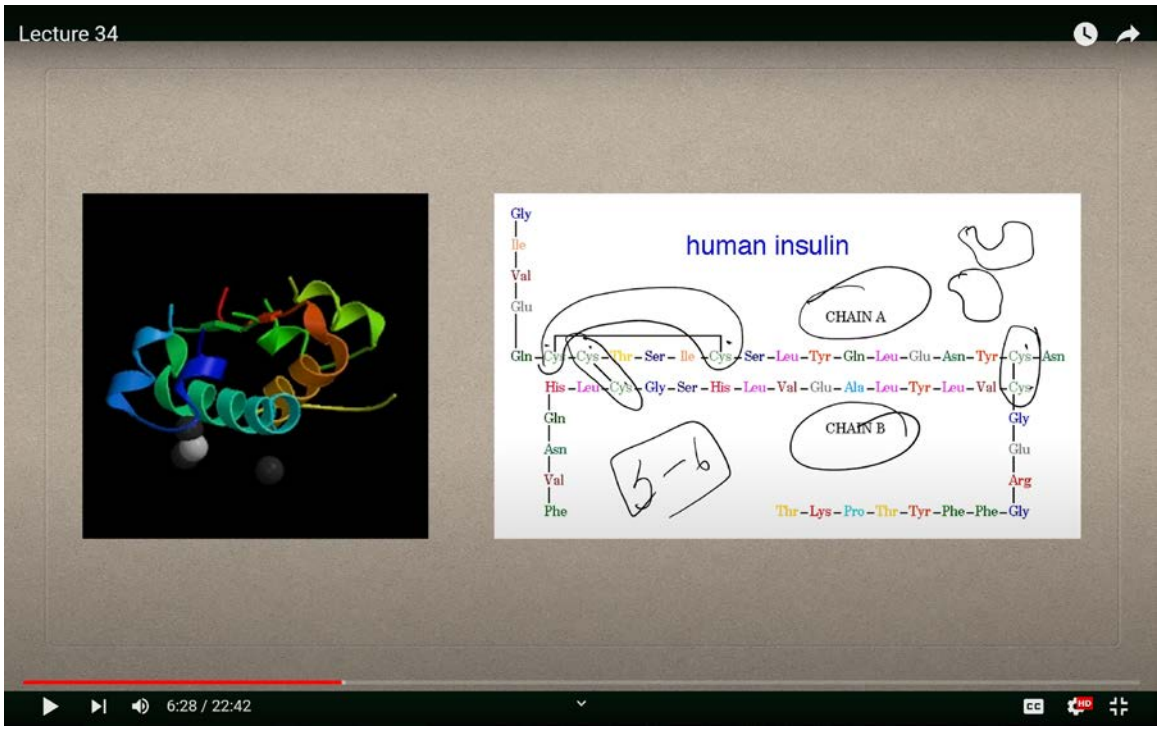

These are the inherent challenges you'll encounter when conducting experiments of this nature. Insulin, when produced in the body, undergoes a transformation that makes it useful. One of the remarkable aspects of insulin is the change in its structure that allows it to function in the human body. Insulin is first synthesized as pre-pro-insulin, which then undergoes proteolytic cleavage, a process of breaking down proteins, into pro-insulin. Finally, pro-insulin is further processed into active insulin. This is a multi-step biochemical process.

When we examine the structure of insulin, we see several critical features. Insulin has three disulfide bonds: two connecting Chain A to Chain B, and a third within Chain A itself. These disulfide bonds are formed by sulfur atoms from the amino acid cysteine, accounting for all six sulfur atoms in insulin. As I mentioned earlier, insulin contains six sulfur atoms, and the only way this happens is through sulfur-containing amino acids, which in this case are cysteine residues. You can see the cysteines here, 1, 2, 3, 4, 5, 6, each contributing to the formation of these crucial disulfide linkages.

As noted before, insulin is composed of two chains: Chain A and Chain B. Chain A is linked to Chain B via two disulfide bonds, and within Chain A, the disulfide bond causes the chain to fold or curl onto itself. This curled structure is of immense significance because it represents the biologically active form of insulin.

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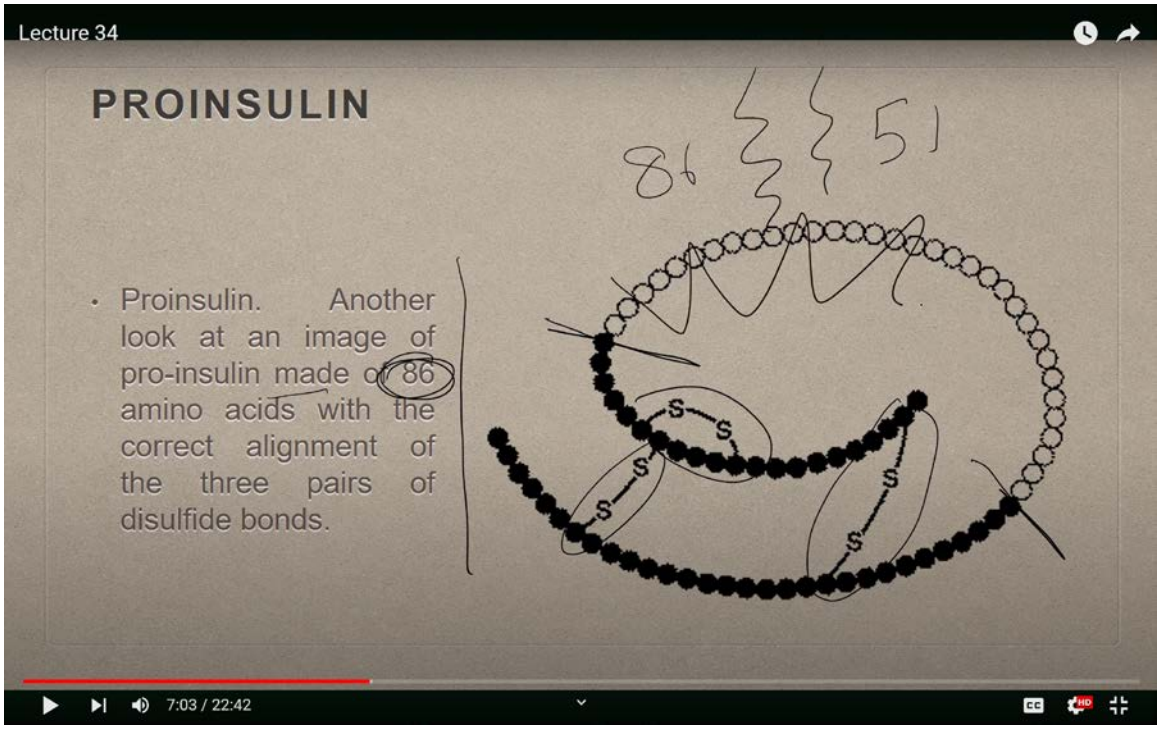

If we shift our focus to pro-insulin, we observe a structure made up of 86 amino acids, significantly longer than the 51 amino acids in active insulin. This difference indicates that certain sections of pro-insulin need to be cleaved for the molecule to become biologically active. Again, we see disulfide linkages in pro-insulin, and the portion that will be cleaved off is clearly identifiable. These are the specific cleavage sites where pro-insulin will be cut, releasing the active insulin.

The process of converting pre-pro-insulin to pro-insulin, and ultimately to active insulin, involves a series of precise steps. Pre-pro-insulin starts as a 110-amino-acid chain. Once the signal peptide is removed, pro-insulin, with 86 amino acids, is formed. From there, the formation of disulfide bonds between Chain A and Chain B creates the backbone of active

insulin. The intervening C-peptide chain is then removed, leaving us with the final biologically active insulin, which contains 51 amino acids.

To emphasize, this is the pathway: pre-pro-insulin is first transcribed as a chain of 110 amino acids. After removing the signal peptide, we are left with pro-insulin, consisting of 86 amino acids. From this stage, the molecule is processed down to 51 amino acids, which is the active form of insulin. Think about the immense precision required here, how fortunate those scientists were to pinpoint the exact active form of insulin, while the other forms remained inactive.

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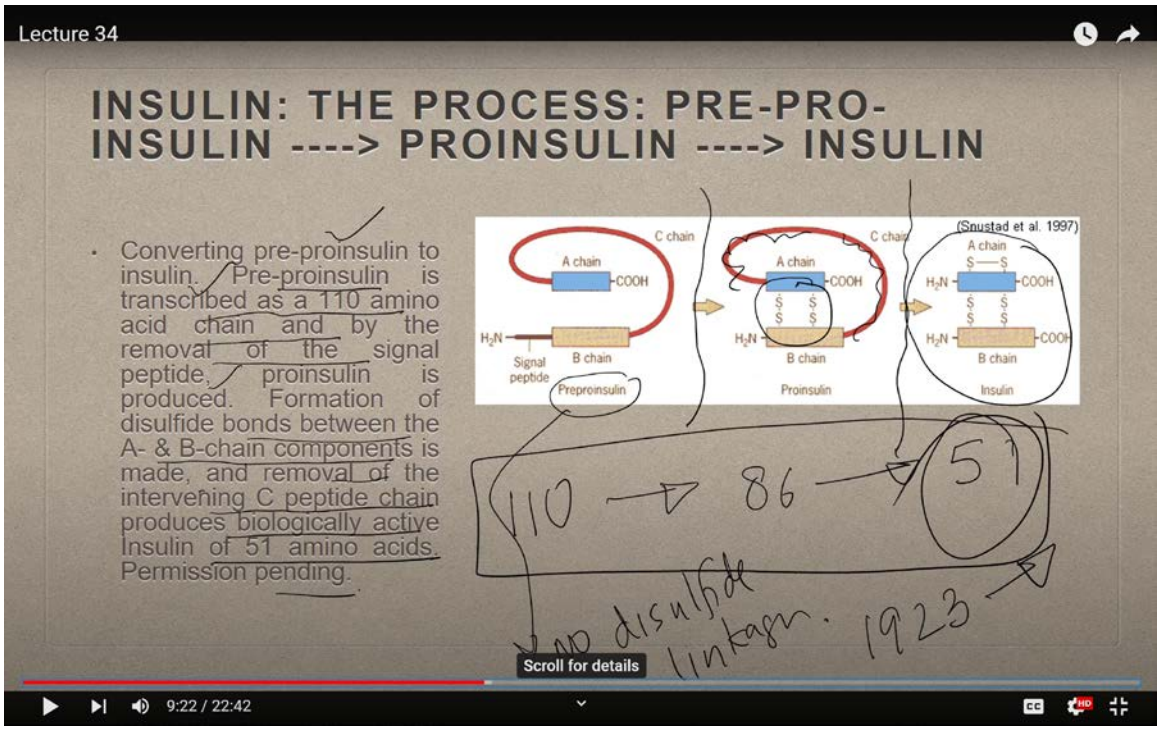

Understanding this conversion from pre-pro-insulin to active insulin is essential. Once pro-insulin is formed, disulfide bonds are created between the A and B chains, and after the intervening C-peptide is removed, we are left with biologically active insulin. This conversion process is crucial to the insulin's functionality and underscores the complexity of producing this life-saving hormone.

At the pre-pro-insulin stage, there are no disulfide linkages present. However, as insulin

progresses through its formation, the disulfide linkages are established, and eventually, the C-chain is removed to form active insulin. It's important to note that much of this understanding came much later, certainly not in the early 1920s. In fact, 1921 to 1923 marks the beginning of our modern understanding of insulin and its use in treating diabetes. Prior to this discovery, the physiological role of insulin in maintaining blood glucose levels was unknown.

Here's how it works: when we consume food, particularly foods rich in sugar or carbohydrates, the digestive process begins. The carbohydrates, which are readily available sources of energy, travel through the digestive tract, being absorbed in the small intestine. These carbohydrates break down into glucose, which enters the bloodstream. Insulin acts as a gatekeeper here, facilitating the transport of glucose from the blood into the body's cells for immediate energy use or for storage as glycogen, an essential energy reserve.

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If insulin is absent, however, glucose remains in the bloodstream, leading to elevated blood glucose levels. As the glucose concentration in the blood rises, the blood becomes denser

due to its increased viscosity. This denser blood puts additional strain on the heart, making it work harder to pump blood throughout the body. The kidneys also face stress, as they attempt to excrete the excess glucose via urine, a phenomenon known as glycosuria, which was one of the early symptoms studied by researchers like J.J.R. Macleod.

In cases of diabetes, the body cannot absorb glucose from the bloodstream due to a lack of insulin. Consequently, the heart is overworked, the kidneys are strained, and the body's energy levels are severely compromised. Under normal circumstances, when glucose levels rise, insulin is secreted by the pancreas to help cells absorb the glucose and reduce the blood glucose level. Essentially, the pancreas acts like a clock, when it detects an increase in blood glucose, it triggers the release of insulin. The insulin then binds to specific receptors on cells, opening the "gates" that allow glucose to enter.

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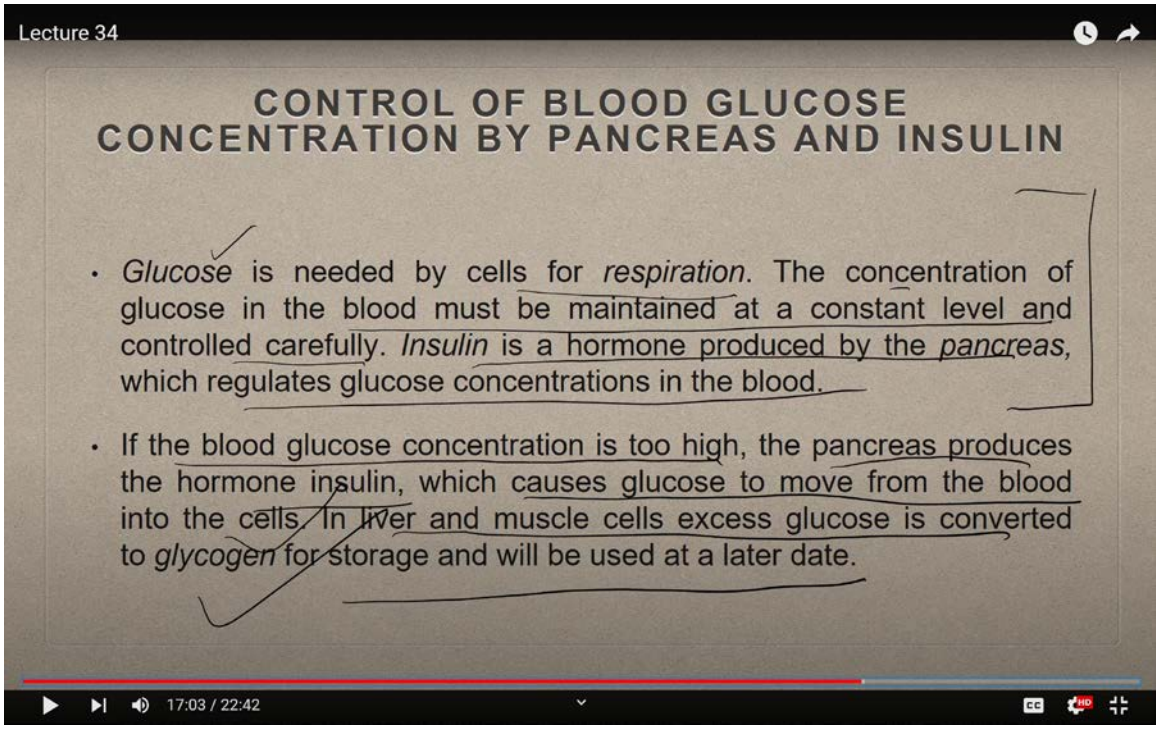

Insulin's role is crucial, it binds to receptors on various tissues and facilitates the uptake of glucose from the bloodstream, effectively regulating blood sugar levels. This is why we often refer to insulin as a gatekeeper: it ensures that cells can access glucose, and by doing

so, it prevents dangerous levels of glucose from accumulating in the bloodstream.

Now, when we discuss diabetes, especially types 1 and 2, we're talking about conditions where this insulin mechanism is disrupted. In type 1 diabetes, there is no insulin production, so glucose remains in the blood rather than being absorbed by the cells. In type 2 diabetes, the body becomes resistant to insulin, meaning even if insulin is present, it may not be able to bind effectively to its receptors and perform its function.

When glucose levels drop too low, the pancreas responds by reducing insulin production, allowing glucose to remain in the bloodstream. Additionally, other hormones, such as glucagon, come into play. Glucagon helps to break down stored glycogen in the liver, releasing glucose back into the blood to maintain energy levels. This intricate balance of insulin and other hormones ensures that blood glucose levels stay within a healthy range, whether the body is in a state of high glucose (after eating) or low glucose (fasting or between meals).

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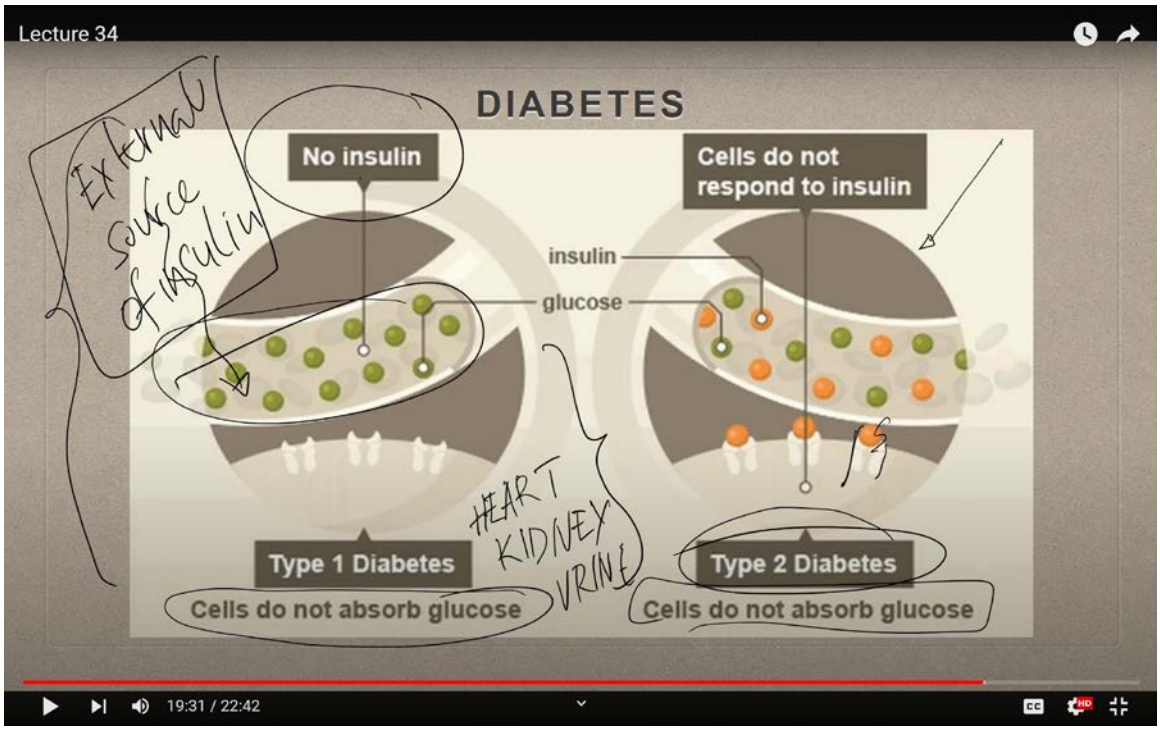

Now, imagine a scenario where glucose levels are low and insulin is mistakenly injected.

This creates a complex situation, which we'll explore in more detail soon. For now, it's important to grasp that insulin is crucial for regulating glucose levels in the blood, ensuring that cells receive the glucose they need for respiration. The pancreas continuously monitors blood glucose concentration and adjusts insulin secretion accordingly. When there is an excess of glucose, the liver and muscle cells store it as glycogen, to be used later when energy is needed.

Without insulin, this vital process breaks down, leading to diabetes, where the body can no longer regulate blood glucose effectively, causing severe metabolic consequences.

If the body ceases to produce insulin, the glucose concentration in the blood rises drastically, leading to a condition where cells are unable to absorb the glucose they need. This excess glucose in the bloodstream significantly disrupts blood flow, causing a cascade of complications. As I've previously mentioned, this affects the heart, kidneys, and even the urinary system. The body begins to experience symptoms like excessive sweating and perspiration as it struggles to cope with the absence of insulin. In such cases, the only viable solution is an external source of insulin, which can bind to tissues like adipose tissue and the liver, enabling glucose absorption and restoring balance.

This scenario exemplifies type 1 diabetes, where insulin is completely absent and must be supplemented externally. However, there is another, more complex form of diabetes known as type 2 diabetes. In type 2 diabetes, the body produces insulin, sometimes even in adequate amounts, but the cells do not respond to it. Insulin, as we know, acts as the gatekeeper that binds to cells, particularly in skeletal muscle and the liver, to open the gates for glucose absorption. But in type 2 diabetes, despite the insulin being present, the cells have become resistant, and the gates do not open. This results in a situation where glucose remains in the bloodstream, unabsorbed.

This form of diabetes is far more complex because it involves cellular resistance to insulin, likely due to some form of mutation or defect at the insulin receptor site. This is a critical distinction between the two types of diabetes: while type 1 diabetes can often be managed with insulin injections, type 2 diabetes is a completely different challenge. It cannot be treated in the same way and requires a different approach altogether.

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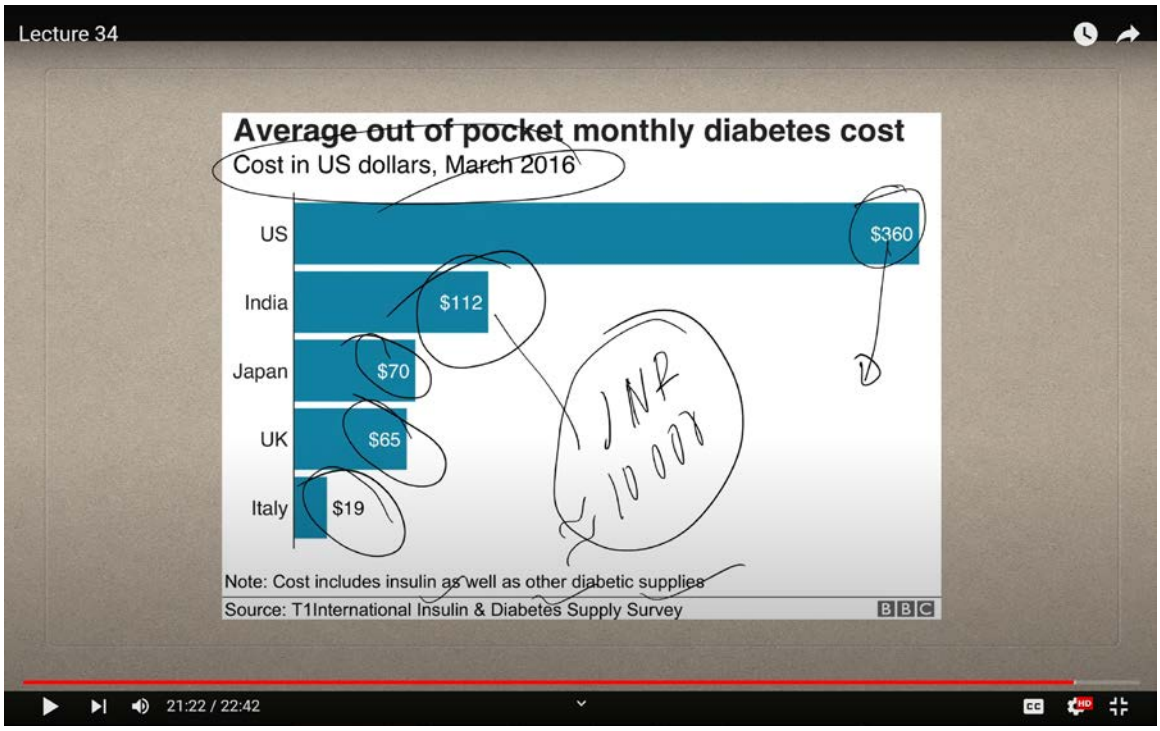

Looking at the financial burden of managing diabetes today, we can see how significant the costs are. In March 2016, for example, the average out-of-pocket cost for diabetes in the U.S., even after insurance coverage, was around $360 per month. In India, this figure was approximately $112 per month, which amounts to about 10,000 rupees, a substantial monthly expense, even after insurance contributions. In Japan, the cost was lower at $80 per month, while in the UK, it was $65. Italy had the lowest cost at $19 per month. These expenses cover insulin and other diabetic supplies necessary to manage the condition.

According to the International Insulin and Diabetic Supply Survey, conducted by the BBC, these expenses reflect the substantial financial burden that many people face in managing diabetes. Even in the U.S., $360 per month represents a significant cost, contributing to the growing global concern surrounding diabetes management.

As we look to the future, it's clear that the prevalence of diabetes is rising, and the challenges associated with managing this condition are becoming more daunting. India, for instance, has become known as the diabetes capital of the world, highlighting the urgent

need for continued research and innovation in diabetes treatment.

In our next class, we'll delve into a darker side of insulin, the history of murders committed using insulin, a grim aspect of its story that has sparked controversy over the years. Thank you for your attention today.