BroadcastMed | Grand Rounds: Role of Circadian System in Regulation of Insulin Secretion: Implications for Diabetes Prevention and Treatment

ADRIAN VELLA: Good afternoon. Thank you for coming to CCATS Grand Rounds. My name is Adrian Vella, and I represent the endocrine interests of regenerative medicine. Anyway, it is my pleasurable task to introduce Dr. Matveyenko. Dr. Matveyenko actually just recently came to Mayo. He came in August from UCLA, where he was on faculty since 2008. He's a distinguished beta-cell biologist. He's establishing a reputation for himself in the field of beta-cell biology, and we're glad to have recruited him with help of donor funding to the regenerative medicine initiative. He has a special interest in circadian biology and how it affects beta cell function. And so I won't take more of his time. It's my pleasure to introduce him, and we look forward to his talk. Thanks Aleksey.

ALEKSEY MATVEYENKO: Thank you very much. So as Dr. Vella alluded to, my interests are in understanding the role of circadian system in regulation of insulin secretion, particularly with emphases on developing a new preventative and therapeutic approach for patients with diabetes. So these are disclosures. I have to disclose that I in the last past 12 months, I have received basic research funding from Takeda Pharmaceuticals.

So as an outline today, what I will first talk about-- basics molecular and physiological mechanisms of beta-cell failure in diabetes, both type 1 and type 2 Then I'll do a brief overview of also molecular and physiological basis of regulation of circadian system. The meat of the talk will be on on some of the studies that are-- some published and some are still evolving in revision or unpublished on the role of circadian system in regulation of pancreatic beta-cell function survival and regeneration, as it relates to diabetes. And I conclude with a final story of-- an example of how we can use chronotherapeutics, which is basically a fancy word to say, how can we use circadian system or circadian medicine as potential therapeutic and preventative approach to treat diabetes and primarily preserve beta-cell health.

So as an obligatory slide to show you the extent of diabetes epidemic. I like to show this slide for two reasons. First, it's outdated, which I kind of like. So what this shows is that prevalence of type 2 diabetes in 2000. And then at that time, estimates of what it will be in 2030. The reason I like to show this is that CDC just released figures of prevalence. And these estimates were already beat. So there are already approximately 30 million Americans with type 2 diabetes. So these estimates were short, and we got there in half the time. I think most alarmingly, again, the same CDC report released showing that nearly 30% of Americans have pre-diabetes or impaired fasting glucose. And this has relevance to the topic.

So to orient everybody to the pancreatic beta-cell, which is the organ that I'm interested in, this shows a cross-section of the pancreas stained for insulin in brown. And you can see the organ that I study is difficult to get to. It's difficult to study, almost impossible to study in humans. And also difficult to study in animal models as well, largely because the cell type that I'm interested in is only about 1% of the pancreas. So shown here, the pancreatic islets of Langerhans. If you

isolate them, just look in them in the phase contrast microscope, these are spherical structures. If you go a little deeper and do immunofluorescence, even the pancreatic islet, which is 1% of the pancreas is not homogeneous itself. It's a collection of endocrine cells--mostly beta-cells, which are stained here in green when the insulin's stained. But there are also alpha cells that produce glucagon, that are also critical for regulation of glucose metabolism. Once you zoom in on the beta-cells, it's a typical secretory cell. It's full of insulin, insulin granules, which is its primary function. And this is also visible at the EM level. Again, this is a cell that's largely full of this insulin secretory granules that are full of insulin as well as other peptides that are secreted.

Insulin secretion mechanisms is fairly well-described. Basically it's a typical metabolic stimulus secretion pathway where we couple increasing glucose concentrations following the meal as all of us are eating right now. Blood glucose concentration rise. Glucose gets transported into the pancreatic beta-cell. They are glued to a transporter, subsequently phosphorylated by high km enzyme glucokinase. And this is very important. These GLUT2 and glucokinase have high kms, so they can respond to elevations in glucose concentrations. This leads to oxidation and production of ATP. An increase in ATP to ADP ratio leads to closure of potassium-sensitive ATP channels, calcium influx via voltage-gated calcium channels, leading to insulin granule exocytosis and insulin secretion. So beta-cell is basically a neuroendocrine cell, which couples increase in glucose concentrations to a electrophysiological event leading to insulin secretion.

So what about diabetes? What are the primarily abnormalities that we see in diabetes in the pancreas? So shown here on top are examples of a representative islets, here stained for insulin in brown in population. So these are cadaveric. This is a cadaveric study actually done in collaboration with Mayo Clinic. Cadaveric samples, islets from a control individual, an individual with impaired fasting glucose, type 2 diabetes, and by contrast and type 1 diabetes. And here, there are estimates of the deficit in beta-cell numbers. So this number beta-cell mass, basically a fractional area of insulin to the total pancreatic section. And what you can see, perhaps alarmingly as I mentioned earlier, even in impaired fasting glucose, there's already approximately 50% deficit in numbers of your beta-cells. This subsequently increases to about 65% in type 2 diabetes. And just by contrast, type 1 diabetes associated with your complete loss of pancreatic fetus cells.

This is attributed and this has been confirmed in a couple of studies with increase in beta-cell apoptosis. So patients with type 2 diabetes, again if you looked at their cadaveric pancreas--

which is the only way we today can get to the pancreas-- there's about a several fold increase in beta-cell apoptosis. There are still some arguments in the field-- and largely it's due to the difficulties in studying this-- whether there are abnormalities in beta-cell proliferation or beta-cell regeneration. In humans, it's almost impossible to methodologically assess that. But increase in beta-cell apoptosis have been reported in a number of studies in humans.

What about beta-cell function? Beta-cell function has been shown to be decreased in a number of studies, including some excellent work here at Mayo. I like to show this kind of a classic study by Brunzell, looking at insulin secretion, acute insulin secretion, in response to intravenous glucose challenge. And what he did here is that he classified individuals by their fasting glucose levels. And again, alarmingly-- and again this data has been also shown here-- that even quite a mild elevation in fasting glucose leads to a substantial reduction in glucose responses, inability of beta-cells to respond to glucose and secrete insulin. And this is almost completely gone in individuals once fasting glucose is elevated.

So we have abnormalities both in a beta-cell mass, largely due to a decrease in beta-cell apoptosis, and there is abnormality in beta-cell function. The field is still divided whether-- what comes first, but it is, I think, unanimously agreed that both are present in patients with type 2 diabetes and of course, type 1 as well.

So what are the mechanisms of these decline-- beta-cell failure? A number of them have been proposed, including genetics. Again, some excellent work of my presenter Dr. Vella has done a lot of work in understanding how genetics leads to susceptibility to beta-cell failure. Glucolipotoxicity, introuterine environment, as well as toxicity due to islet amyloid polypeptide aggregation. This is an area that I've spent some time studying, and I'll use some of these models in my talks, so I'll just briefly introduce it in a couple of slides.

One other feature of beta-cell loss in type 2 diabetes is that when islets lose their insulin expression in type 2 diabetes and lose their beta-cell mass, it's typically associated with increase in amyloid aggregation. This is a stain for Congo red. This is amyloid stain. In the same study, looking into a large cohort of cadaveric pancreas, the prevalence of amyloid in the islets of patients with type 2 diabetes is anywhere from 80% to 90%. And this is associated with aggregation of this islet amyloid polypeptide. This islet amyloid polypeptide is about 99% homologous to a beta polypeptide that you're all probably familiar with Alzheimer's disease.

And it's fairly conserved in number of species, interestingly, except rodents. Rodents actually

have proline residues in the region that gives it it's amyloidogenic or toxic propensity. So one of the supporting evidence that this plays a role in beta-cell failure in diabetes was to develop animal models where you can introduce the human version of islet amyloid polypeptide. And we've done that. We've developed rats and mice. And this is data from a human amyloidal polypeptide transgenic rat, where this human IPP was overexpressed on the insulin promoter. And as you can see, we can nicely, we capitulate pathology of the islet that you saw it in patients, that there is less insulin expression, there is amyloid aggregation, shown here by Congo red staining, and there's increase in beta-cell apoptosis, shown here by TUNEL staining, which is a marker of cell death. Importantly, these animals with age develop hyperglycemia. It takes them a while, which is an interesting fact. Similar to humans, they develop hyperglycemia with middle-age for a rat. They lose, progressively, beta-cell numbers, beta-cell mass. And they also lose insulin secretion.

So how does this fit into the regenerative medicine? And the question is, why am I here, right. So this is a nice slide-- thank you to the Center of Regenerative Medicine-- kind of providing the basis of regenerative medicine. You want to repair tissue. Cardiac tissue, islet tissue, basic tenants of it-- rejuvenation, regeneration, replacement. So looking at islet rejuvenation, it'd be important to improve beta-cell function. And something I'm not going to talk about today, glucagon, but glucagon secretion is also impaired in diabetes. And it's very important so I put it here. In terms of regeneration, obvious, if the data is correct, if the data really holds true, we have a third of the population that's probably deficient in numbers of beta-cells. And this deficiency could be as high as 50%. So there needs to be understanding mechanism of stopping beta-cell attrition as well as increasing beta-cell formation. And replacement is development of beta-cell replacement strategy, either using IPS cells, embryonic stem cells, adult stem cells, as well as technology, artificial pancreas. And all of this work is happening at Mayo.

So what my interests are are how the environment contributes to this triad. In particular, my recent interest in understanding how the circadian system, the regulation of circadian system, can contribute to beta-cell function regeneration. So the first kind of a little conclusion to the first part is that beta-cell failure in diabetes is characterized by loss of beta-cell numbers and their function. And this is attributed to intracellular accumulation of human islet amyloid polypeptide oligomers.

So briefly introduce circadian system, which will be next of my talk. Circadian system is

perhaps one of the most conserved physiological systems in our organisms. Organisms anywhere from unicellular bacteria to mammals have the ability to change their internal metabolism in response to changes in the light-dark cycle. In mammals, the master clock of the system is located in the suprachiasmatic nucleus of the hypothalamus. So in response to light, the specialized retina ganglionic cells in our eyes detect changes in the light cycles. And these cells are actually different from rods and cones.

So we have specific sensors in our eyes that can respond to changes in light-dark cycles. They relay information to the SCN. SCN subsequently can synchronize clock genes or clocks in most of the tissues of our body. The way it does it, it's still not really known, but it's likely mediated via activity, body temperature regulations, as well as neuronal or hormonal signals. One example is melatonin-- a hormone that's secreted in the diurnal fashion-- has been hypothesized as one of these mechanisms that SCN, our brain, can then train clocks in all of our in all of our cells.

So what is this clock mechanisms? Basically it's a transcriptional translational feedback loop that's regulated in a 24 hour cycle. We have two key transcription factors, CLOCK and BMAL, that typically at the beginning of the day, they heterodimerize and activate transcription of negative feedback activators, typically PER and CRY. There are a number of others, and I'm just simplifying it for this talk. PER and CRY translocate to the cytoplasm. There with time, it gets ubiquitinated and similated, and it can actually feed back and inhibit BMAL transcription. And this loop is happening 24 hours, pretty much, in every cell in our body.

Importantly is this is the way that our cells have designed to regulate transcription in this 24-hour basis and synchronize our intracellular transcription with the changes in light-dark cycle. And this is done typically by BMAL controlling what's called clock control genes. And they regulate a number of processes in our body, and estimates range. Anywhere from 5% to 20% of all the genome is, under this clock, regulated.

In the beta-cell, it's not known. This is one of the areas that I'm very much interested-- as how important this clock is actually regulating transcription in the beta-cell and I'll show you some data shortly. So how do we disrupt this? We can disrupt the light input. I think all of us are pretty good at doing that by working long hours or shift workers. You can also disrupt it at the level of the clock genes. So the genetics play a role.

So what are the evidence that circadian disruption predisposes to type 2 diabetes in humans?

There are three lines of evidence. Epidemiological studies. So a number of studies have shown that sleep loss and shift work increases your susceptibility to type 2 diabetes. Important to know that shift work, particularly, increases susceptibility to number of diseases. And actually recently, it's been qualified as a potential carcinogenic as well. So it is one of the areas of interest, but again, these are epidemiological studies, and they just show associations.

Some groups have tried to experimentally address this question. Again, this is very difficult in humans. But groups have tried to model shift work in a laboratory condition and look at the effects on metabolism. And most of this work comes from Scheer, from Harvard Public Health, showing that taking young human volunteers and exposing them to shift work-like conditions for as little as a week can increase glucose excursions after breakfast, lunch, and dinner.

And then genetic studies, so mouse studies where you can manipulate or disrupt the clock, the whole body. One of the primary abnormalities in these animals is that they're hyperglycemic. They get diabetes.

So just a final slide to point out, again, some of these human studies. The same group, to me, is the only study that actually looked at beta-cell dysfunction. They've taken human volunteers and they exposed them to 21 days of this shift work-like conditions. So they misaligned the time that they sleep-- so the time they sleep, they eat-- with external environment. And they kept them here for three weeks. And then they subsequently looked at the metabolic abnormalities. Interestingly, the glucose intolerance-- which is shown here in red-- so they give them an oral glucose drink. They were glucose intolerant, and then they had a significantly decreased insulin secretion. So this is one of the key abnormalities of this period. So they could disrupt beta-cell function by misaligning their internal circadian system with external environment.

Interestingly, following a week of recovery, it normalized. So it's obviously a functional effect, hopefully not an effect that affects beta-cell survival or mass.

So another way to look at the system is in engineering terms. You have input. You have an oscillator, which is our suprachiasmatic nucleus. It subsequently synchronizes all of our tissue oscillators. And then we have an output, hormonal secretion all the way to molecular rhythms within ourselves. So I'll use this paradigm to demonstrate some of the basics of circadian system.

Let's look at sleep-wake cycle. So this is how we study this in the lab. I mean, it's much easier

to control in animals. We have animals that-- we can control their behavior. We can control their light-dark cycle. And as output of the circadian system, sleep-wake cycle is the most robust. So what this shown here is actigraph. So dark is the dark cycle. Light is the light cycle here. Each line here is a day, and here you can see animals. These are nocturnal animals, are active at night. And they're sleeping during the day. They're again active at night. There's a robust 24 hour period. OK?

So let's look at this to demonstrate two basic characteristics of circadian system. These clocks are synchronized to light, to external stimuli. And this clock persist or free run in temporal isolation. First the light. You take these animals-- here they are running during the night-- and then you flip the light-dark cycle by 12 hours. As you can see, almost immediately, animals, their SCN isn't trained to the different light-dark cycle. So they synchronize to the light almost immediately. So that's the feature of circadian system. If you put them in a complete dark-dark, complete isolations, it's important to know that although they don't have the cue, they still maintain that near 24 hour period in activity. So they just get up a little later every day, but they still maintain a 24 hour cycle. So these clocks are untrainable by external stimuli for the brain that's light. But this clock, if you put them on isolations, the transcription continues in a 24 hour cycle.

OK, what about molecular rhythms? Do these clocks persist in islets? So how do we study this? A basic way is to simply sacrifice animals. You obviously cannot do this in humans with pancreas, but people have done it with adipose tissue and other cell types that you can actually extract from humans in a diurnal fashion. In due quantitative PCR, you see that our BMAL gene is cycling in opposite fashion with a period gene. So these are the two positive and negative feedback regulators. But to longitudinally do this, the best way is to introduce a luciferase reporter in one of these clock genes. So this is done. This is a rat that's transgenic for period one luciferase. So a clock gene in this animal, in this rat, has a luciferase reporter. Then you can subsequently isolate pancreatic pilots, put this on the [INAUDIBLE] cycle. And using bioluminescence recording, you can actually look at the diurnal changes in this clock gene expression by measuring changes and bioluminescence. OK? [INAUDIBLE] movies.

So this is about 15 islets in culture recorded during the three day period. And then they isolate their clock gene expression by-- you can pick it out by measuring bioluminescence. When you do this, you can zero in on individual pancreatic islets, and you can see that they have nice diurnal isolation phases. OK? So their clock gene expression is in a 24 hour cycle, even taking

out of the body. So it's persistently running as it would be with, let's say, a sleep-wake cycle. When you compile them all together, all these islets, taken from the same animals in culture, still have the phase of their clock oscillations. They're nicely in phase. The amplitude is different because they're different size islets-- they're different amount of cells-- but they're all nicely in phase.

And you can quantify this. You can look at the amplitude. You can look at the period, which is near 24 hours-- here it's about 23 hours for islets-- and the phase, when the expression is the highest. Then you can start asking questions. Do islets respond to light? Obviously the don't, right? But something is propagating the signal from the brain to the islet.

So if you do the same experiment when you change light-dark cycles 12 hours, animals changes its activity. You isolate their pancreatic islets, the phase of clock oscillations in the islets changes with activity. So there is communication from the brain all the way to the pancreatic islets as you change your activity in the light-dark cycle.

So what is the signal? One of the signal that does this in the liver is feeding. So feeding is one of the ways that our circadian system can entrain the clocks in our body. So when we did this experiment, we kept the normal light-dark cycle, but we restricted their feeding to the light period. So we shifted their feeding six hours. Animals typically start feeding at the onset of the light-dark cycle, and we shifted it to middle of the light cycle. When we do that, we isolate their pancreatic islets. We can shift their clock gene expression almost exactly the time that they shift their feeding. So the clocks and islets are entrained by light, light entrained in the brain, and then entrains the feeding. And the feeding is really what's driving the clock gene expression in islets. OK?

Most importantly, does this does this matter for islet function or the things in all survival proliferation, all the important factors that we are interested? And just to give you a little example, one of the ways we're approaching this is doing genomic analysis-- you know just measuring diurnal rhythms in gene expression in pancreatic islets, looking at, using chips sequencing, looking at targets of these transcription factors.

As an example, this is 11, I think, sorry, 10 or 11 key genes regulating insulin secretion in islets. So here, animals were sacrificed at four hour intervals during the light and dark cycle. And you can see there is nice cycling of insulin secretion regulating genes, particularly with the trough during the day when the animal is sleeping, and the peak at night when the animal is

supposed to be active, responding to feeding. When you introduce all 51 key genes regulating insulin secretion, you start seeing nice diurnal patterns. You can superimpose genes regulating proliferation, for example. Those genes, interestingly, are anti-phase with insulin secretion. So these are the type of studies we're doing, and they're still qualitative, and still not answering the question-- does the clock really regulate beta-cell function in survival.

So coming back to the next question here is, so what is the role of circadian system in regulation of beta-cell function in survival and in regeneration in diabetes. Well there are two ways you can address this. And you know-- as scientists typically do when they want to figure out if something is connected-- let's disrupt one system and see if there's an effect on the other system. So the way we can disrupt circadian system, we can disrupt the light input, global circadian disruption, or we can disrupt, specifically, the clock in individual cell type that we're interested in.

So I'll show you some data from two models. OK? One is rat model-- for more physiological studies, our lab likes to use larger animals, rodents. So we have rats where we can alter their light-dark cycle and look at their effects on their beta-cell function survival in proliferation. And we have a mouse model where we can use a Cre-lox system, and using tamoxifen, selectively delete BMAL, which is our key transcription factor in the beta-cells. So this model has normal global circadian rhythms, but the clock, just in beta-cells, is completely demolished. So they have no cycling of clock genes. To illustrate this in activity level, here is our normally running animals. It's active at night, inactive during the day, and active at night. Here's our light-disrupted animal. So if we introduce shift work-like conditions, animal has no diurnal rhythms. And here is our animal that has clocked disrupted just in beta-cells. So it's global circadian rhythms are absolutely normal, but the beta-cell clock is diminished. And we can [INAUDIBLE] them.

So what kind of studies we can do with this is we can use this. We can look at the model of global circadian disruption to see what are the effects on the pancreatic beta-cell clock. So if we isolate pancreatic islets from these animals-- so these are LD and LL disrupted animals-- as you can see, and these are PER-luciferase oscillations from individual islets-- as you can see, the amplitude of these oscillations declines. They still have a period. So even if they're exposed to circadian disruption, they can still maintain the period. Their phase is diminished. Their phase is completely disrupted. So they're no longer in phase with each other, and the amplitude of transcriptional isolation is diminished.

So the relevance here is to perhaps, the shift work nurses that are disrupting their circadian system daily, what this does is disregulates clock gene expression in their pancreas, and I'm assuming in other tissues as well. We can look at this model to try to identify-- so what are the changes, transcriptional changes in response to deletion of the clock in the mice.

So when we do this, for example, we can look at our beta-cell specific BMAL-knockout mice. So this is the mice where clock is [INAUDIBLE] in beta-cells. And we look at the global gene expression during the light cycle for control in our clock deficient mice and during the dark cycle. And I think even visually, you can see there are a number of changes in the gene expression, just by selectively removing the clock. And interestingly, you know nearly 80-- I mean this is the informatics data that you get-- but nearly 80 signaling pathways are significantly altered once you remove the clock from the beta-cells. Some of the notable ones, DNA replication, insulin secretion, apoptosis. And interestingly, protein processing-- very important for regulation of survival in beta cells-- is probably the most significantly altered once you disrupt the clock in pancreatic beta-cells.

So what about predisposition to diabetes? I'm going to summarize, in a slide or two, a number of studies here. But basically, our approach was to-- OK, now we know we can disrupt the clock even globally or selectively in beta cells. Let's introduce a metabolic stressor such as diet-induced obesity or increased expression of human islet amyloid polypeptide to stress the system and see how these animals respond metabolically. So shown here are glucose levels, fasting glucose levels, which is a readout for type 2 diabetes in some of our studies. During the 10 weeks of either normal light cycle or our disrupted LL dark cycle, in normal lean animals, not much is happening. Right? So just a slight increase in fasting glucose levels. Once you introduce diet-induced obesity-- or you do this in animals that have this genetic susceptibility for amyloid aggregation and cell apoptosis-- you start seeing a significant increase in glucose concentrations.

So the clock becomes more important for susceptibility to type 2 diabetes, at least in animal models, once you introduce a metabolic stressor such as obesity or IAPP overexpression. If we look at IAPP animals, for example, at the beginning, I showed you that it takes these animals about five months to get to these impaired fasting glucose hyperglycemic levels. We were able to do this with circadian disruption in a couple of weeks. So it's quite a significant stressor to a system. But it is important to keep in mind, these are animal studies and results here are often exaggerated. So once we look at in this model- so these are human IAPP

transgenic animals, they are susceptible for beta-cell loss-- if you expose them to circadian disruption, you increase beta-cell loss-- shown here by loss of insulin staining-- and you significantly increase beta-cell apoptosis.

You can do CLEM studies and look at insulin secretion, insulin sensitivity. And the primary effect is the loss of insulin secretion in response to a hyperglycemic CLEM. So these animals lose their insulin secretion. However, they retain the insulin sensitivity that they have-- again consistent with some of the data in humans where the primarily defect of circadian disruption appears to be beta-cell function. What about our mice? So again, this is the model where beta-cell clock has diminished just in beta-cell. Similarly, just lean beta-cell knockout mice, despite there are 80 signaling pathways that are altered-- that our genomic analysis tells us-- the fasting glucose levels remain fairly consistent. However, once you introduce the stressors, such as obesity, you start seeing a significant increase. Interestingly, in these animals, primarily beta-cell effect is actually an ability to increase their beta-cell mass in response to increase in metabolic demand.

So this is an example of an islet from a lean control animal. And if you introduce obesity in a lean animal, the beta-cell mass expands. And it expands by increasing beta-cell proliferation. This expansion is altered in these animals. So there is now a deficit-- not just the function-- but the ability of beta-cells to enhance proliferation. And a lot of our interests are in trying to understand the mechanisms and what specific pathways are affected here. So to summarize our almost final area, is that the global disruption of circadian rhythms as well as loss of beta-cell clock gene expression pairs glucose control, function, and beta-cell formation. This is particularly happening in the context of metabolic stress.

So finally, I wanted to-- in the last, maybe 10 minutes or so-- give you a little example of how circadian system can be used as, perhaps, a therapeutic agent. OK? So again, coming back to our engineering model, is that as you describe circadian rhythms, you also disrupt the ways that SCN is communicating with other cells. And I mentioned that melatonin is one of the hormones that's been hypothesized to be one of these main entrainment agents in our body. Melatonin is a hormone secreted at night. Interestingly, in humans and in rodents and most animals, it's a nightly secreted hormone from the pineal gland and has long been known to regulate circadian rhythms, specifically entrained these tissue circadian clocks in our body.

Interestingly, in recent years, people have started to hypothesize that it may have a independent effect on cardiovascular system function, immune function, metabolic function,

and type 2 diabetes. Most importantly, melatonin is suppressed by light. So its secretion is proportional to the light intensity. And this is shown here from a very old study, but it can see that as light intensity increases-- as your exposure to light intensity and specifically the blue light intensity, which is what these ganglion cells and retina sense-- the percentage suppression increases. So one good way to decrease your melatonin secretion is to watch TV before you go to sleep or look at your iPad. We can talk about it during our Q&A.

So once you discover something exciting, you start looking through literature, and you realize that people thought of this long, long before. And I thought this was interesting-- just digging this up at the library-- saying that as early as 1957, it's been hypothesized that melatonin may be related to regulation of beta-cell function. Some other evidence here is that a recent study from JAMA-- again looking at a large population of nurses-- they've been able to correlate-- it's a correlative study-- the diminished melatonin secretion is associated with a twofold increase risk of type 2 diabetes, again epidemiological. Perhaps the most direct evidence that melatonin secretion or melatonin receptor activation may be related to beta-cell health in type 2 diabetes comes from genetic studies. In 2009, there was a series of nature genetic papers showing that a common variant in melatonin receptor is associated with impaired insulin secretion type 2 diabetes. And melatonin regulates cyclic AMP-PKA pathway. This is the path very critical for regulation of insulin secretion.

So let's talk a little bit more about melatonin receptor variance. Just a quick search of PubMed-- since then, nearly 72 papers, all confirmatory showing that this variance in melatonin receptors associated with type 2 diabetes and largely insulin secretion. This is the this is the figure from original paper showing the disposition index, which is a good measure of beta-cell function, is progressively decreasing with increase a variant in human population. And also recently, it's been shown that it's indeed the loss of function variants that increase odds ratio of development of type 2 diabetes. Accumulating evidence suggesting that perhaps activation of melatonin receptor signaling, in itself, may be important regulation of beta-cell function.

So we wanted to address that. So how does melatonin receptor-- how do they signal? Interestingly, it's a basic GI-coupled receptor. So there are two, MT1 and MT2. Interestingly, in response to acute melatonin activation, which is about less than two hours, it decreases cyclic AMP, protein kinase A, phospho-CREB pathway, a basic biochemical pathway on all the cells. Interestingly, prolonged exposure to melatonin-- something that occurs throughout the night in people and in animals-- once melatonin is withdrawn, this pathway is actually super sensitized.

So once you withdraw melatonin and then activate this pathway, the activation of PKA in phospho-CREB in cells is actually enhanced. And this has been shown largely in pituitary cells. And phospho-CREB is important for beta-cell function. It directly phosphorylates insulin mRNA, as well as important for pro-survival and pro-functional gene expression.

So we wanted to address this in beta-cell. This is staining for melatonin receptors in human and rodent beta-cells. It's very pronounced. I don't know if you guys have seen it. It's highly expressed. It's expressed in beta-cell lines. Once you expose cell lines to melatonin, this receptor internalizes, which is kind of a typic function of a G-coupled receptor. So it's there. Next, we wanted to use cell lines since to see, OK, is this pathway actually sensitized in beta-cells as well. So we looked at beta-cell lines, and we did a fairly simple study. We looked at phospho-CREB expression at baseline in response-- a basal. And it responds in two key activators of this pathway in beta-cells, encroaching peptides GIP and GLP-1. These peptides, not only activate this pathway, they are also very important for insulin secretion as well as regulation of survival and proliferation. As you can see, if you pre-treat cells with melatonin, there is an enhanced activation of CREB at baseline and particularly in response to GIP and GLP-1.

As a control, we can introduce phospho-ERK pathway, also activated by GIP, GLP-1. There is no enhancement in the mean data shown here. So what melatonin does-- at least in beta-cells, as it's shown in other cell types-- it enhances sensitivity of the beta-cell to activation of cyclic AMP-PPK pathway, particularly in response to activators such as GIP GLP-1. Once you look at the gene expression-- so then we can collect the genes and we can see that at the gene level, the Cre-enhancer sequenced genes, the ones that are regulated by phospho-CREB, significantly enhance in cells pretreated with melatonin, whereas ERK pathway is flat. So there is this specific enhancement of phospho-CREB activation in beta-cells in response to melatonin.

Then we can take the same cells, we can overexpress human islet amyloid polypeptide. This is a cell model now of cell stress. These cells have increased cleaved Caspase-3, which is cell apoptosis marker and phospho junk, oxidative stress and ER stress marker. And melatonin pretreatment can decrease cell death markers in these cell lines. This is also associated with decrease in oxidative stress, measured here by [INAUDIBLE] method, which is a method where you can measure total carbonyl addition to proteins. So here, in a cell line at least, we can see that pretreatment of melatonin enhances cyclic AMP pathway. And it can, at least in

cells, improve survival of the cells. What about islets? So we do get human islets. So most of our islets-- actually non-diabetic human islets that we can get shipments. So we can test some of these hypotheses, specifically, does this alter function. Which is all that we care.

So we have to make these human islets type 2 diabetic. So we do that by incubating them for 72 hours in high glucose, which is a glucotoxicity model. So this is a busy slide, but I'll walk you through it. So this is basal and glucose stimulated insulin secretion in control islets incubated at low glucose, five millimoles per liter. If you incubate human islets for 72 hours in high glucose concentration, they lose glucose responsiveness, largely, that their basal insulin secretion goes up, and it's flat. They aren't glucose responsive. Pre-treating islets either with melatonin or melatonin receptor agonists-- so basically, what we did here is we put our islets to sleep every night. We gave them a melatonin-- we introduced melatonin in the media for 12 to 14 hours, kind of recapitulating the night cycle. And in training these islets to their regular cycle, you can see we can retain beta-cell function. GLP-1 stimulated insulin secretion showed a similar pattern. Interestingly, melatonin wasn't, at least significantly, improved in in GLP-1 stimulated insulin secretion, but receptor agonist did that.

We have also shipments of islets isolated from patients with type 2 diabetes. And we saw a similar pattern here. Patients also show diminished beta-cell function, so a loss of glucose responsiveness. And it can be improved with melatonin or receptor agonist. Here, just the variance was too high to show significance. And similar pattern was shown with GLP-1 stimulated insulin secretion. Although, again, because of this, I think this is end of four shipments. So because the number of replicants here are not as high, some of the statistics weren't able to be shown.

So I think I'm at least exactly at 12:45. So final slide. So then again, we can take these type 2 diabetic islets, treat them with melatonin and melatonin receptor agonist, and we can do some genetic studies to see what pathways are altered. Interestingly, perhaps the most notably down-regulated pathway with melatonin receptor treatment is-- surprise, surprise-- our protein processing and endoplasmic reticulum stress pathway with markers like CHOP, JUNK, all being diminished in response to melatonin receptor activation.

So in summary, first I wanted to give you some idea of a mechanism of beta-cell failure in diabetes, largely that it's characterized by the loss of beta-cell numbers and their function. I introduce circadian system and show you that it synchronizes behavioral, physiological, as well as transcriptional rhythms in our body in response to changes in light-dark cycle. Showed that

global disruption of circadian rhythms, as well as specific beta-cell clock disruption impairs glucose control, beta-cell function, as well as beta-cell formation. And this particularly happens in the context of metabolic stress. I used obesity and human islet amyloid IAPP overexpression. Expression And finally, circadian activation of beta-cell melatonin signaling perhaps presents a potential therapeutic approach to preserve beta-cell function in diabetes.

I would like to acknowledge most of this work, actually all of this work-- my lab is almost done-- so all this work was done at UCLA, the Division of Endocrinology there and also in collaboration with their Circadian Medicine group, led by Chris Colwell and Gene Block. I'd like to thank the people that worked. These are Jingyi, which is the graduate student in the lab that just passed her quals. Kuntol and Anthony Thomas are the postdocs. Sofia Costes is another investigator at UCLA that collaborated with me on the INS cell work. And I would like to acknowledge mentorship of Peter Butler, who was the director and division chair, and has been a mentor for me there and continues to be. And funding for this work come from NIH as well as Larry L Hillblom foundation. And Regenerative Medicine for bringing me here. I'm excited. Thank you very much.