Episode Transcript

Danny Lennon: It's great for us to be here. We've been very much looking forward to this. We have what we hope will be something quite different and quite interesting lined up. And the goal is not only just to tell you about some facts about nutrition, but as you probably know from hopefully listening to much of our content of... when we come to looking at research and how that informs our decisions, how to actually go through that process. And so as I described in the email that I sent out to you, one way is to the research paper that you are all sent. We're going to mention that, but we're also, what we're trying to do is not say "this is *the* research paper we are putting all our conclusions on".

(Rather) it's... number one: what interesting topics does this bring? What do we know about this already? What might this add? And then what conclusions do we come to afterwards? Because this is the issue. We want to know over time, how do we continue to update our knowledge? And so hopefully you've seen, if you gave that paper a quick read that there are some detailed parts, some parts maybe that don't make some sense, maybe some conclusions that are difficult to come to.

And hopefully by the end of our first session today, some of that will be a bit more clear. And then after a short break, we're going to have a couple of

sessions lined up that will hopefully be useful when you're going to try and read research yourself and some ideas to be aware of. And then hopefully during the q and a, because we have a nice space to do this, we're going to be able to get to your questions and hopefully have something to offer with our discussion.

The paper we sent you out was related to glucose tolerance, melatonin, circadian rhythms, chrononutrition, that we've mentioned before. And so as a way to maybe start this it might be useful to get some background context before we get to this actual paper. So there's a couple of concepts you may have heard us mention previously on the podcast or if you've ever looked at stuff around meal timing, chrononutrition. For example: what is circadian misalignment? How does this impact things like eating?

So maybe as a way to start, I'm going to put it over to you, Alan, seeing as Alan has direct expertise in this area off the back of his doctorate, with, first of all, why is this a thing of when it comes to eating, why do we think of this in terms of timing, what this does physiologically to the body? And then what is this idea of circadian misalignment?

Alan Flanagan: Probably the first real sense that nutrition was going to have potentially an important interaction with circadian rhythms or this concept of circadian alignment likely came from observations in shift workers, night shift workers in particular, that there was a profoundly increased risk of cardiovascular and metabolic disease in night shift workers that was often independent of factors that we would expect to mediate that relationship like BMI.

And although it's difficult to disentangle exactly, because night shift work will encompass a range of risk factors. It's not just eating during the biological night, it's the fact that someone has extended wakefulness, sleep curtailment and this disruption that is akin to jet lag. So isolating the effects of food in that context can be difficult.

But there started to emerge a line of experimental research laboratory based, very tightly controlled studies that would manipulate someone's light exposure and their meal timing. And that started to show that actually meal timing per se, was really important in people's glucose tolerance, insulin sensitivity, and in their capacity to have more efficient metabolic function.

So I think in order to try and understand why the timing of someone's eating across the day could be important, it's important that we do have this concept. And this graph is a nice illustration of this, of what we would mean by a circadian cycle and a behavioral cycle. So from the circadian side, Basically that's the term, the word itself, *circadian* is a Latin derivative from "around the day".

And the early research in this area was actually conducted in Germany a researcher named (Jürgen) Aschoff in the 1960s. And he took his students, it's a fascinating body of evidence, and basically put them into disused bunkers, which were completely pitch black. So there was no light input, there was no clocks, there was nothing that could give the participants a sense of time of day.

And what he noticed was once they were in that environment for a number of consecutive days, total darkness, that if you measure their melatonin or their core body temperature, that the rhythm, that the rhythmic phase of that melatonin or core body temperature was longer than the 24 hour day.

And that's because we're diurnal mammals; we're day active mammals. And so left to their own devices with no time cues, these rhythms will run longer than 24 hours in most people. There is some differences where it can run shorter, and so in our normal environment then we have these different cues, time cues, the light timing of the light cycle during the year, which changes obviously the timing of the dark cycle, and they're not timing of behaviors as well.

So when we have those behaviors aligned to the underlying circadian cycle, our circadian rhythms synchronize exactly to the 24 hour period of the day. And those rhythms will follow that repetitive pattern. We can map them out and we can see them fluctuate nicely from one day to the next. But of course, that's our underlying biological rhythm.

We then have our behavioral cycle, and that's often in dependent on us. So circadian alignment as a concept occurs when our behavioral patterns are in sync with our underlying rhythms in circadian biological processes. And our misalignment occurs when we alter that. For example, if we start to suddenly have a pattern of behavior where we're awake till 3:00 AM, then that's a disruption to the system.

Now the system is adaptable and flexible, which is why if we fly from here to Sydney, you'll be a little bit messed up for a few days, but you will adapt to that new timing, that new light dart cycle, and your behaviors will become in sync with where you are in that new time zone. So we do have the capacity to get over jet lag. But from a nutrition perspective, what's been an interesting question is, putting extreme situations of circadian disruption aside, like shift work or jet lag when you fly a long way east or a long way west, putting those scenarios aside, what's the potential impact and relevance of less extreme but more consistent disruption to the system? So if we're living in an environment where our circadian rhythms expect to operate on, what would be our local day and night cycle, what's the effect if we superimpose our behaviors onto that with, for example, delaying when we eat, distributing energy later in the day, having a large proportion of daily energy in the biological evening I'll explain what we mean by that versus just evening time.

And so all of these questions. Have become more relevant in light of a lot of observational research that associates that pattern of eating with late night energy intake and breakfast skipping with increased risk of type two diabetes, metabolic disease in particular. And then we have experimental studies that suggest that not just the underlying circadian rhythms in these processes have a rhythm that we know.

For example, with glucose tolerance, it's really well established that independent of other factors, your capacity to uptake glucose into cells and to have a strong insulin response is enhanced in the morning time, in the early part of the day and decreases in the evening time. So we know that underlying circadian rhythm in glucose metabolism exists.

And then we have this other experimental research that then suggests if there's this bulk of. Energy in the evening that will lead to adverse metabolic outcomes. However, there have been inconsistencies in this research that have led people to say it's probably just either "#calorie deficit", it's probably just a pattern of other negative behaviors. The reason we think this study is interesting today that we can get into is because it provides us, perhaps with a link to explain some of the inconsistencies in the evidence as it relates to clock time versus biological time, which I think we can get into.

Danny Lennon: Sure. So maybe as a way to recap that and to get into this topic a bit more, a few things. So one of these is a graph from an old paper by

a researcher, Frank Sheer, who you may have noticed is one of the authors on today's paper, and just speaks to this idea of, as an noted that we can get these different responses in postprandial glucose. So what our blood sugar does after eating, depending on if we have this circadian alignment versus misalignment. So that's one way to look at it. One of the also interesting things that we've just discussed that might be interesting about today's group is that the lead author Marta Garaulet has done a lot of work in this area, and her group have proposed a way to reconcile some of that disagreement you mentioned in the area.

So for example, there's her, some of her work originally said, if we have high levels of melatonin, this could be one of the things that's making glucose tolerance worse at night. Of course then there's disagreeing research on this; there's research suggesting otherwise. So her group has been one to put forward an idea they call like the "timing model", which explains that it's the concurrence or the overlap between both levels of melatonin and then your timing of food.

And so as on a very basic level, what this means is yes, one scenario that could lead to this worse glucose tolerance could be when you have, when you're eating during the middle of the night. And we'll expand on that in a moment because during this time is typically when you have these high melatonin levels and that could cause this worse glucose tolerance.

But also if someone had for whatever reason elevated melatonin other parts of the day and were eating at those times, that could similarly cause those problems. So as a way to explain this a bit more in relation to either any of this concept from their lab or just the how melatonin links in here, what is the best way and the simplest way to conceptualize this relationship between meal timing and melatonin and any other interesting areas about melatonin that we should start with?

Alan Flanagan: Yeah, so there's two definitions that I think we need to nail down now. Before we get into this study, and it's this idea that we typically, and even in research now you'll just see "time" referred to, or "timing", but from a physiological perspective that can mean different things. So we can have "clock time", which is, right now it's 13.26 (1.26 pm) , that's the clock time. But in each individual here, there will be an underlying biological time. So let's assume that we're all being exposed to the same light dark cycle.

Right now, we're all relatively aligned to a similar time zone here, but let's assume now that it's the evening, what we would call the evening, and if I was to measure Danny's melatonin and my melatonin, this is purely hypothetical, and let's we define the biological night as a time at which melatonin has elevated above certain thresholds. So because melatonin is primarily responsive to light cues, to light exposure, we have a really quite elegant pathway of detecting light with our eyes, with retinal cells. And we have this very specific pathway of relaying information about light from our eyes to the central clock in the brain.

And that helps to regulate melatonin. So everyone's rhythm will, will have the same type of shape, but the precise phase or timing of when that rhythm is higher or lower in individuals can. And that's largely genetic, although it's also influenced by age and sex. So if I measure Danny's melatonin and we got what we would term his "melatonin onset", and let's say hypothetically it's 11:00 PM and then we measure my melatonin levels and my melatonin onset is 8:00 PM. So we could both be sitting there at, let's say, for example, 9:00 PM clock time, but our underlying biological time is slightly different. I'm in my biological night because my melatonin levels are now elevated. Danny's still in his early evening because his melatonin hasn't reached that threshold yet. So this is why we have differences in what we call chronotype. Some people that are more aligned to and early, they would get up earlier, want to go to bed earlier.

We get people that go to bed later, want to sleep. And the reason this is crucial from a metabolic perspective is, as Danny said, this timing model that Marta Garaulet and colleagues have proposed suggests that it's that elevation in melatonin and the interaction between and the timing of food intake and that elevation in melatonin that likely explains the adverse metabolic effects of eating at night.

And the reason this is crucial is because most research will only consider clock time. But if you decided we're going to test two different meal times, we're going to go into test 6:00 PM clock time, and we're going to test 9:00 PM clock time, and you've 20 people and you don't know what their underlying biological time is, what their melatonin rhythm is, then you're going to get different responses because those mealtimes are going to occur at a different proximity or phase relative to that elevation in melatonin in each individual. And if you actually go back to that slide from the Scheer study... So this was

the glucose response. Now this was using a very extreme based protocol where they basically did the type of jet lag that you would get if you flew from here to Sydney; this 12 hour flip. But you can see like purple (line on the graph) here: purple here had no change in their glucose in response to that really extreme shift. Whereas red and green were basically up where you would expect someone with pre-diabetes, serious metabolic disease to be, even though these were otherwise healthy young, lean participants.

And so one of the theories is that some of this individual variation could be explained by melatonin and melatonin receptor. So it's really important. So when I'm referring to clock time, that's literally the time you would look at on your watch right now. But if we're talking about biological time, what that means is we're talking about the actual underlying rhythm in melatonin when that melatonin is elevated.

And the importance of this is that it's, and this is the hypothesis being proposed by these researchers it's the interaction of food intake with that elevation and melatonin that is what is primarily responsible for the adverse metabolic effects of eating, either in the evening for people that are early types or even if we get into say, a shift work scenario.

At that point, almost everyone's melatonin, apart from a really extreme, about 3% of outliers who would not have their melatonin elevate until two 3:00 AM clock time; very late chronotypes. So this likely explains some of the inconsistencies that we might have in the research if it's only looking at clock time. And also it potentially provides us with an actual explanation mechanistically for some of the adverse effects of eating at night.

Danny Lennon: So we have this difference in when that melatonin is going to rise. So this happens across the day. We all have this diurnal variation, but when that starts to rise, may differ from individual to individual. And I think this brings up one marker that people may have heard in different research papers that we should explain now. And this is this idea of dim light melatonin onset: DLMO. And this is used as a way to, rather than look at clock time, we're going to look at someone's dim light melatonin onset and look at how much of their calories are they eating closest time or when are they having a meal after this dim light melatonin onset. Is there anything about DLMO that we should mention here that might be useful to add some context?

Alan Flanagan: Yeah, I think one, from a research perspective, DLMO is difficult to do. We measured DLMO in a lab study that I did as part of my PhD and it involved taking blood samples every hour in people from about 3:00 PM till four in the morning.

Danny Lennon: And they didn't like that? (sarcasm)

Alan Flanagan: There were tears on the ward that I was not prepared to handle.

Danny Lennon: Some of them yours!

Alan Flanagan: Thankfully we had some fantastic clinical nurses that were on hand that I was running into being like, "I dunno what to do here. This is human emotions".

So it's really difficult to do, even if you're going to do a field study and you want to measure DLMO, you're going to have to get your participants into a lab and stick a needle in their arm and get blood every hour until four in the morning. So I think there, that's why it's not obviously as used as often as it could be. There are ways that you could try and get people to do it in the home, but basically that involves them waking themselves up every hour and spitting into a tube, which you can imagine the compliance is but the reason DLMO is such a robust measure is because if you do get that amount of samples from someone, you can chart, you can map out their melatonin increasing.

There are ways of calculating what DLMO would be. And basically it's like an average of a certain amount of measurements above a certain threshold, right? So a lot of the time people will just use about 10 picograms per milliliter of melatonin if it's from blood samples or three sorry, three milligrams if it's from blood 10, if it's from saliva.

And so basically you're taking this threshold of melatonin and you're saying once melatonin's above that threshold, that's an indication that's their biological night. And the reason this measure's really important is because it gives us the most robust assessment of that individual's circadian phase.

And the reason that's important then is because then we can start to actually understand how the timing of whatever intervention, what we're doing, or whatever exposure we're looking at, if it's observational correlates with elevations in dimlight melatonin. And we can individualize that data for each person.

And the reason this is important is a couple of the studies Danny just mentioned from this group at Harvard. Andrew McGill was the lead author on two papers. And what they did was, it was a cross-sectional study, but they did, they brought participants into the lab and they measured DLMO in all of their participants. Then they had the participants go about their daily life for a week using an app to track their dietary intake. And then what they did was they analyzed their distribution of energy, both related just to clock time and related to biological time to DLMO in each participant. And what they were doing was they were looking at it cross-sectionally where they stratified participants according to levels of body fat.

So they had lean participants who had body fat of around 22%, which I guess is lean for the general population. And the other group had body fat percentage of around 34 to 35%. So they were categorized as obese in terms of body fat percentage. And what they did looked at was how did these two, the lean and overweight/obese groups differ in the timing of their food intake?

And when they just looked at it relative to clock time, they didn't see any associations. But when they looked at it in relation to biological time, when they looked at it in relation to individual's proximity of food intake to their nocturnal rise in melatonin, they showed quite a significant association between the proximity of calorie intake to DLMO and higher body fat levels.

And they also used a metric, which is the midpoint of caloric intake, which is if you're doing observational research, this is quite a nice way of getting a single representation of someone's pattern of energy distribution. So you're taking their dietary intake across the day. You're calculating what clock time did they reach 50% of their daily energy intake. So obviously if someone reaches that at 5:00 PM clock time, that means 50% of their energy is coming after that. And if someone reaches it at one o'clock, it obviously means they've reached that point much earlier in the day.

So what they found was that the closer that midpoint of energy intake was to DLMO to the nocturnal elevation in melatonin, the higher the body fat percentage in the participants. So these were two studies, 2017 and 2019. Again, both cross-sectional, both observational, but they actually, for observational research lent a huge amount of weight to where the direction of this research has been going. Because the main take home point was if you're just thinking about clock time, you're you may be missing associations and it's likely the biological time factor where each individual is slightly different. And the more that people have energy intake in close proximity to their own biological time.

Which can differ, like I said, hypothetically Danny's might be 11:00 PM mine might be 8:00 PM So our proximity of food intake is going to be relative to that, to those respective times. So if I have DLMO at 8:00 PM and I eat a big meal at 9:30 PM I'm going to have some pretty adverse metabolic responses to that meal.

But if Danny's DLMO was 11:30 PM for example, and he ate a meal at say 9:00 PM he's possibly going to be okay because it's well in advance of when that nocturnal elevation. Even though if we were looking at the clock time, we'd be like late night eating. So it might not be the clock time that's important here. It might be individual biological time.

Danny Lennon: One final thing to maybe connect this to some previous discussions we've had and previous work from this group. Just an overview of one of the papers from Marta Garaulet's lab was some of the work that has looked at this distribution of calories that you've probably heard us mention on the podcast before.

And rather than just thinking of timing, where most of those calories are distributed across the day is that, for example, front loaded where there's more earlier in the day versus a larger proportion of calories later in the day. And it's a lot of this works group this labs work that has looked at differences here.

This is just one example you're seeing on screen where they had people that consume a later or earlier largest meal of the day. So for some context, they typically do this in populations in the south of Spain where their larger meal is the middle meal or the lunch meal as opposed to the final meal.

And they looked if people had that earlier or later in the day. And based on the proportion of their daily calories, are there differences in things like glucose tolerance? And so we're not going to get into this specifically, but this is a way to connect some of what we've discussed previously in relation to not looking at just what times of day do people eat, but as a percentage of their total daily calories. Where are those placed? And so I think that's something you've often identified as an area, not just again, clock time or even biological time. But front loading or back loading caloric intake.

Alan Flanagan: Yeah. I think that's important because the time restricted feeding research, for example, largely is quite underwhelming a lot of the time. And some of the studies are just real duds, but that's another topic. But the point here is that even with these factors we're discussing, there is still an underlying circadian rhythm in glucose tolerance. And some of the findings in the study, when we get into the specifics of this will highlight this, that there's still, if we take people we've researched going back to the late eighties showing this, really tightly controlled lab studies.

If we take otherwise healthy people, even independent of their melatonin rhythm and independent of, say their, the actual calorie and macronutrient content of a meal. If we feed people the same meal, same calories, same macronutrients at 8:00 AM versus 8:00 PM we will typically get better glucose responses to the 8:00 AM meal versus the 8:00 PM meal.

Now, in otherwise healthy individual. The magnitude of that difference might be relatively modest, but in people with metabolic disease or on that beginning of that glucose intolerant spectrum, then the differences become increasingly larger and more relevant. And that's because there is this underlying, what we call diurnal variation or variation according to time of day in glucose tolerance, where it's amplified for various reasons in the morning versus the evening. And then we layer on these additional considerations like the concept of biological night or evening versus clock time. And obviously then some of the genetic factors that we'll discuss.

Danny Lennon: Great. So the final thing maybe to mention before we actually get to the study is maybe one of the more complex things of that paper that you may have noted is this discussion around a specific risk variant.

So in particular this MTNR1B gene, which is associated with type two diabetes, and this is what is, at least in this paper, suggesting this link with melatonin and glucose metabolism. And in particularly, they look at a certain risk variant for this that is listed out that has this correlation with glucose fasting glucose, as well as maybe responses to that.

The only thing that we really need to know for our purposes here, as opposed to anything in detail, is really that we have these two different alleles here, a G or a C. And we can think of the G simply as the risk allele, and then C as the non-risk one. For example, we can have CC, CG or GG . In the CC we don't have any of the risk allele present, so would have lower risk in this situation compared to if we have someone with one of these G alleles, which is the risk version of that.

And I think they mentioned that about 30% of the European population may be carrying this particular allele. So all we need to know for interpreting what this might mean is that we have G representing a risk allele here. And then C being that non-risk one, and this may change that relationship.

Audience Member: Risk for what?

Danny Lennon: So this is a type two diabetes in particular.

Audience Member: Risk for type two diabetes later in life or generally?

Alan Flanagan: Just elevated general risk. Your baseline risk of diabetes is higher with the GG.

Audience Member: And the CC? Not at all?

Alan Flanagan: No. No, it's not. And the reason for this is, so the MTNR1B is short for melatonin receptor. And so while everything I've said about melatonin so far has really been by reference to the light dark cycle, and that's how most people think of melatonin. If we say it, we think if you say melatonin supplement, most people will think sleep supplement. If we think of melatonin, will typically relate it to sleep.

But it's a pretty fascinating hormone. And melatonin receptors have been identified throughout the body, including in the pancreas. And importantly

for this concept of circadian rhythms and biological day versus biological night or just the light cycle. And the dark cycle is we basically have distinct phases physiologically, right? All animals do, pretty much, certainly all mammals do. Where in humans, for example the light phase of the day is our waking phase, it's our active phase, it's our feeding phase. And the dark phase or the night correspondingly is the opposite of all of those typically. So it's the sleeping, rest, digest, fasting phase.

And so we need these environmental contrasts for circadian rhythms. But importantly, part of the reason why, and we still don't fully understand the mechanistic link of elevated melatonin, it might be to do with the interaction with the pancreas. We're still not quite sure mechanistically what this relationship is and how it's acting.

But the theory essentially is that, look, once melatonin is elevated, this is our biological night. This means physiologically, melatonin is signaling throughout the body that this is, that they can expect that the body, which is, this is all an anticipatory machine right now. Once melatonin's elevated, it's signaling throughout the body "Hey, it's nighttime, it's rest, it's sleep, it's digest, it's fasting".

And then we throw food intake on top of that and the system goes, what are you doing to me? . So this is at the most basic distillation why there might be this association with elevated melatonin. It's because physiologically the body has shifted from this signal that melatonin has given us into our biological night where it's not expecting food intake, it's not expecting to be awake, it's not expecting to be active. And then if you've got a genetic predisposition to higher glucose anyway, then it could obviously be something that. is impacted then by these interactions.

Danny Lennon: Yeah. Because I think they did mention one of the potential mechanisms here was that with this risk variant, you get greater expression of that melatonin receptor at the pancreas specifically. So that's just one potential way. So we can think of that generally as our risk variant there.

So maybe let's actually talk about some aspects of the study. So this is part of an ongoing study called the ONTIME-MT, which is just one of these acronyms that they use as an ongoing study. This is one publication that's came from it. We're looking at a randomized design here, over 840 adults. And you can see

some of their baseline characteristics here. And again, a couple of things to note. Again, similar to this lab's previous work, this is a population typically in the south of Spain.

So again, it has some in interesting characteristics when it comes to eating because of the timing of meals there, and also where that they place an all of that intake. So within these particular participants related to that, those distributions of the risk allele, they noted that 10% of people were GG; so both alleles being that allele. 40% were CG and then 50% were CC. So they're able to compare along those lines, first of all. And then each person in the study underwent this different intervention that was a simulation of early and late feeding. I think they outline it here. We have basically an oral glucose tolerance test that's going to happen early and then also late for each person.

And this is to simulate what would happen in, for example, if someone's eating early or late. And so what this means, as you can hopefully see here on the screen, is that after this eight hour fast, they have people then do an evening time oral glucose tolerance test, which is 75 grams of glucose they're consuming.

And then with measurements for two hours afterwards, the early condition that you see on top is scheduled four hours before their habitual bedtime. So this is relevant to typically when that person would have their bedtime, four hours previous to that is going to be that "early timing" and then one hour before that habitual or normal bedtime is the "late timing".

And if you look into some of the tables in that study, you'll see that on average for the early condition, this is going to correlate to about 19.50 in the evening. And then for the late condition that is about 22.50 at night what would correlate to those early and later habitual bedtimes.

We have this oral glucose tolerance test conducted washout period, and then they do the opposite condition. So this is our basic setup here to mimic this earlier and later feeding. And what we're of course, trying to look at then is possible responses. So maybe let's start working through some of the initial results and then we can get into some of the interesting implications from that.

So the first thing we see here is in relation to glucose. So particularly on the right hand side, we have this glucose area under the curve for earlier and late, and we see a distinct difference here. They know this is about 8% higher during the late condition, and they noted that the fasting glucose was similar, but the post load, so this would be the same as a post-meal glucose levels were higher in the late oral glucose tolerance tests. So based on some previous work, maybe nothing that stands out immediately from there. Anything you wanted to note on the glucose results, or shall we run through them all first?

Alan Flanagan: No, I think just to, so you get a sense of, we discussed a lot running into this about this idea of clock time versus biological time. And so this study was obviously actually administering these meals, even though it was one hours and four hours before bedtime in the early and late conditions respectively, that one hour was relative to the individuals circadian phase. And that's really important. So just to give you a sense of how wide ranging this could be with the four hour before bedtime condition, the actual timing of that meal in each individual ranged from 6:00 PM to 11:00 PM. So that's the range that's reflecting the differences in across each individual.

And then in the one hour before bedtime, that one hour before bedtime, the timing of that meal ranged from 9:00 PM to two in the morning, 2:00 AM. So this is giving you a sense of actually, if we just think about clock time, we'd say, oh, we gave them a meal at 9:00 PM but if we're thinking about biological time, we're getting this big spread of difference that reflects each individual's melatonin and their biological time.

Danny Lennon: Yeah. So that's our glucose. One thing quickly to note then on the insulin response, we see in the late condition, we get this decreased insulin response and we'll maybe mention why that is important. And then in relation to the risk variance, if you see in one of these tables here if you can't make it out, it's completely fine. All we're seeing here is that there's essentially this dose dependent increase in how the glucose area under the curve changes with late compared to early. And so increasing number of those G alleles. So in other words, going from CC to CG would mean increased risk. And then if your GG further increased risk again, in terms of or further increased glucose area under the curve based on which of those combinations you have would change what that response was.

And then we can see this mapped out in some of the charts and findings from the study where again, you see here the genotypes are listed on top CC, CG and GG. And this is our glucose responses with the early being, the light colored line, and then the dark colored being the late timing. And we see some differences there. And any first things to note here that are important?

Alan Flanagan: So this data is really interesting, I think at two levels because yes, we can look at this and we can see that there's this effect of the genetic variant. Okay? So we can see that in this, what we'll just call this risk type people with this genetic risk type in the melatonin receptor. Yes. You can see that pretty early on in that late condition. It's just much higher, right? Their actual postprandial glucose level is way higher. But even if we look at the non-risk or the normal genetic variant type, you'll see that the late eating is still, now, it doesn't emerge over the first 30 minutes postprandial, but it does start to then emerge from about this 60 minute mark.

And these last three time points are significantly different whereby this late eating condition is significantly elevated. So this is consistent with what we know about this diurnal variation in glucose tolerance, such that even almost independent of genotype, we're seeing that the late eating condition still results in higher glucose compared to the earlier eating condition.

And then when we add the genetic factor on top, we're seeing that in that risk genetic variant than late eating is just much worse. Basically, the magnitude of that response is much more impaired. So I think this is actually quite important because we can focus in on the genetic factor, which is really important.

But actually this study is really in a group of 845 participants, whereas a lot of the lab studies, because of how controlled they are and they need to be, are quite small. So if you remember that graph I was pointing out earlier, the two, that study was one of the first to suggest there was a really adverse effect of circadian misalignment on glucose tolerance.

But it was eight participants and the magnitude of the difference was driven by two participants in terms of their change. You had loads of people just didn't really have much change, whereas this is a really large sample in an nicely controlled trial where we're still seeing that late eating relative to early

eating results in impaired glucose tolerance and then the genetic factor exacerbates it even further.

Danny Lennon: Yeah, and that's key because we can look at something like this where we see that change in glucose here, you see it along the bottom, mapped out in differences of those genotypes. And one interpretation could be, look for someone with this GG combination, the two risk alleles. This is putting them at this worst glucose response to late eating. Therefore, it's a problem if you have this. Whereas as Alan just noted, we see even when you have both of them being C, so that this lowest or non-risk version, you'll still have this worse glucose response in later eating, even when you have technically the best chance of a good response based on your genetics. So there is still some difference. And what this is nice as Alan just outlined, is that it can show us when we almost control for this genetic variation by looking at people with that lowest risk, we're still seeing some degree of difference.

So that's something interesting to note. One of the other things we see then from this is one of the supplemental tables just showed insulin area under the curve that you see that there was interaction between this gene and the dinner timing. And then when we map some of this out in terms of insulin, we see particularly in this GG phenotype, you see here that this lower insulin response based in the late evening group, which again has implications for how someone's going to be able to handle glucose and particularly what their blood sugar is going to do if they have this worse insulin response later on in this meal Timing. Anything to add with insulin?

Alan Flanagan: I think so. In terms of interpreting this as a whole, as far as glucose metabolism goes, when we see this combination of elevated glucose but lower insulin levels, that's communicating to us impaired glucose tolerance.

So you've got an elevation in blood glucose, which in a healthy context should be matched with appropriate insulin secretion to bring that glucose back down into normal range. But we're seeing that, particularly in this genotype, we're just seeing that not happen. So they're getting in both conditions over the first 30 minutes, an insulin response, but then what we would call this second phase, that lasts over the course of the whole two hour period is much lower because the pancreas is basically struggling to

produce and secrete the required amount of insulin for the level of glucose in the blood. So now I think that's an important one because sometimes people will look at a paper and be like "but glucose is up, so how is insulin down?" We interpret that together; elevated glucose, impaired insulin response to mean postprandial,glucose intolerance.

Audience Member: Postpranidial response... for you it must go down in two hours or three hours?

Alan Flanagan: I mean in a healthy response to an OGTT. Absolutely. If we're using that method of assessing glucose and we give someone an oral glucose tolerance test with 75 grams of glucose, we would expect in healthy individuals that by two hours that would be largely metabolized.

Audience Member: And with a CGM (continuous glucose montior) ?

Alan Flanagan: No, this would be using blood tests. You can use CGM. It's a little noisier, but yeah. Most OGTT tests will use blood tests in this case.

These time points are the timing of the blood samples. So you would take it at baseline and then you just have a catheter in their arm. And then thirty, sixty, ninety, a hundred and twenty, you're taking a blood sample. Okay. And then you're mapping that and you're calculating this area under the curve, which means you're taking whatever the fasting baseline was and you're just calculating everything above that.

So it's a representation of the, the total exposure to glucose that would be in your blood or to insulin that would be in your blood for that two hour period. And so this combination, I think is quite important because, again, When we talk about the time of day variation in glucose tolerance, it has a relationship with insulin.

We, we have more efficient insulin responses in the biological morning. And that's related to other factors like GLP one and GIP, which are known as incretin hormones. And basically they, in the, they have a circadian rhythm where they're most active in the biological morning, and they basically enhance the insulin response.

So if you look at people in the morning, we would expect that if we had morning data here, we would expect a healthy insulin response to actually shoot up quite quickly. And that's not a negative, that's a good thing. That's a really strong first phase response. But then we would expect it to come down quite sharply because we've got that insulin out and we're, it's pulling blood glucose back down.

Whereas again, we're looking at the biological evening here and we're actually seeing that it's just staying, even though the response is impaired. It's staying just elevated. And even at this two hour mark here in, in pretty much every in, if you look at the non-risk variant here, and if you look at the light gray bar, you can actually see that in the early condition it's started to come down.

But again, it's probably reflecting the actual the time at which that occurred. So what would be cool if a study like this actually compared not just this late and early biological evening eating, but did the same with the morning as a control, and that I think we'd really see quite pronounced differences.

But yeah, I think this is important to contextualize that this and this may relate to melatonin, right? Part of the mechanistic thinking. And we don't have great data on this, but the hypothesis is that because of melatonin receptors in the pancreas, if melatonin is elevated, , then you're basically getting an impaired ability of the pancreas to secrete insulin in response. So you're not getting the insulin response you need, and as a result, glucose is staying high.

Danny Lennon: One thing I think might be useful to clarify, because we've mentioned insulin response a few times, is this first phase and then second phase insulin response and how that might be applicable to understanding some of these responses.

Alan Flanagan: Yeah so the first phase insulin response is typically what occurs over the first 30 minutes. And that's an immediate response to glucose in the blood. And then the second phase response. And what's interesting is that the progression across glucose intolerant states to a diagnosis of type two diabetes is primarily characterized by a loss of that first phase insulin response; that ability of the pancreas to immediately respond. To detecting glucose in the blood. And then the second phase, so the first

phase, think of as a really immediate sharp first responder. And the second phase is a more prolonged sustained secretion of insulin that's in a healthy context, proportional to what's present as far as glucose in the blood.

And again, brings down into normal range. So if you don't have an adequate first phase response, you can still be secreting insulin in the second phase, but it's nowhere near sufficient to deal with the level of glucose that's there and that glucose is already elevated and contin either continuing to elevate or remaining. Because there wasn't the presence of that first responder response for want of a better analogy.

Danny Lennon: Yeah. One of the things that they added, which was nice in some of the supplemental material was this corrected insulin response, which is a way of representing that first phase insulin response to alland just outlined here in response to glucose.

And the findings are similar to what we've just reported for almost everything else that we're seeing. Number one a worse outcome for late versus earlier feeding. And then that also is exacerbated by what genotype someone has here as well, going from with GG being the worst in terms of this first phase instant response, and then also for the late outcome. So this is just one way of representing that first phase instant response, which was nice.

One of the final things to that we want to get to is some of the stuff on melatonin. So what was also nice in addition to looking at genotypes was then they stratified people by high and low melatonin levels based on the median melatonin in that group. So they took the late evening oral glucose tolerance test, looked at the average melatonin, and then saw who had high and low melatonin relative to this. And they have a few nice charts that you can compare. So what you're seeing here is for a high melatonin concentration in late evening, as measured by that glucose tolerance test. Based along these genotypes, we're starting to. Again, greatest risk here with GG compared to CG. And then very similar responses with the CC genotype. So we're seeing pretty strong connection there. But there's only nuance that we need to add on the this particular graph.

Alan Flanagan: No not particularly. Again I think it's just to note that some wider research that has really dug into the correlation has found that the longer that melatonin is elevated, the more impaired insulin sensitivity. And

some of that research has even looked at, early waking, where you're having a meal at what would be, say 5:00 AM or 4:00 AM. So there does appear to be quite a strong correlation between the elevation and melatonin itself and the degree of impairment in insulin sensitivity and glucose tolerance.

Danny Lennon: So this is then the graphs for glucose. And similar picture here that even having one of those G allelles increases that glucose response. And then with GG is the greatest difference between the early and late as well. And then these were those that had the low melatonin concentration. And the difference here is only really viewable with people with GG. So in general, those with on average, the lower half of their average melatonin concentration.

If those had, GG, you're seeing this distinct difference, whereas CC and CG seem to be relatively similar in this particular finding. So again, we'll get to some of the implications of this, but those were the findings based on high and low melatonin that are worth maybe keeping in mind. So with some of those results in mind, I think what we really wanted to do was, okay, after reading through a paper like this and looking at some of these findings and seeing how it's interesting what we do about number one, coming away with some conclusions from that.

And then second of all, how do we fit that back in with the background context that we discussed earlier? I'll kick it over to you to start as Alan on your first read through of that paper and try to think about the significance of some of these findings. What were maybe some of the most interesting points that we would take away from this specific study?

Alan Flanagan: Yeah. I thought the first for me was that idea that this was a large randomized control trial. That independent of the interaction with someone's genetic predisposition was really confirming that there, there would be a quantitative difference in your glucose tolerance and insulin sensitivity between eating, in this case represented by a glucose tolerance test in close proximity to bedtime, such that the closer that someone would be consuming energy intake in proximity to bedtime would lead to worse outcomes relative to someone consuming that say, four hours in advance of bedtime.

So I think on one level it provided a degree of strong replication and confirmation that, independent of genetic factors, calorie intake or energy intake in close proximity to bedtime is unlikely to be a great idea, certainly over the long term.

And then secondly, I think it added to our understanding of, although the the ecological validity of this is gets a bit more challenging in terms of how we take it away maybe as practitioners and think about real world application. But it really did show that relationship between melatonin and glucose tolerance and insulin sensitivity. And remember this is endogenous, internally produced melatonin. They weren't supplementing people. There are other interventions that have looked at the effects of melatonin supplementation and you can induce insulin resistance. You can induce these responses in people with high doses of melatonin supplementation. 10 milligrams is the dose typically used in those studies.

So I think it really shows that there is some interaction. What exactly is explaining it? We need further research to tease out, but what I've said, it gets tricky from the point of ecological validity or real world application, is that most people don't know what their DLMO is, right? However, the measurement of DLMO and someone's internal biological clock correlates really strongly with the best validated questionnaire for determining your chronotype, your diurnal variation. Most people inherently know whether they like to get up early in the morning or go to bed later at night, right?

Most people inherently have a sense of their own body clock for want of a better term. So I think that this is something that we actually can consider in terms of real world application is just simply you questioning someone or working through with someone what their actual personal preferences, their time of day preferences are, and then thinking about how that relates to their dietary behaviors and their distribution of energy.

So they were the the three main points I came away with. Yes, we're seeing that eating in close proximity to bedtime, to someone's habitual bedtime is not likely to be a good idea for glucose tolerance over time. That independent of genetic variants, that could likely impair glucose tolerance over time and increase someone's risk.

That yes, we're seeing this effect of melatonin evident in both genetic variants and confirming what we've seen in other studies using supplemental or exogenous melatonin. And then finally, that there actually is the potential for some of these findings to really be important for a lot of the associations we see in terms of late night eating, adiposity, and impaired metabolic health.

Danny Lennon: So to build on something you said, and I think this is trying to get it into something practical and useful, is that rather than think about arbitrary cutoff times of, "oh, we can never have food after 6:00 PM" it's more about thinking for an individual that is going to be, it's going to differ from person to person. This is based on that melatonin response, which we're probably not going to be directly measuring in most people, but we can have a few average or looking at averages and associations to come up with some practical guidelines. As an example, Alan noted that in general, if you left people to their own devices, what time they would typically go to bed habitually, we can see that melatonin is probably going to rise, maybe what, two, two and a half hours before that as an average.

I guess there can be some difference, but as an average before that time. And then as an also noted, in general, we see those responses to things like glucose start to come and normalize within a two hour period as well. So working with these, then we can start saying, okay, rather than a specific clock time, depending on when this person usually goes to bed, when they are, when we can guess what maybe their chronotype is going to be, or even use a questionnaire to find that out.

We can now have a period of let's say, three hours as a rough arbitrary number that would give enough time to mean that we're not going to have the peak of that glucose coinciding with a really high level of melatonin. And that's one practical way that is not very, doesn't need a lot of advanced things to do but is also probably ensuring that we're not having the worst of both of these, of really high melatonin and really high glucose load, particularly in people with greater risk, whether that's insulin resistant or otherwise.

Alan Flanagan: Yeah. And again, part of the challenge with a lot of these genetic studies is whatever about not knowing your own DLMO or someone else's, Is, you're certainly not going to know whether they have the GG

melatonin 1B receptor gene. I think what findings like this also demonstrate is that practical takeaways, like the one Danny just outlined will likely benefit individuals in any respect in that context, even if they did have that genetic variant. I, and one thing that I would add for the practitioners amongst you, the way to tease that out isn't to ask someone what time they go to bed and get up at on work days.

The way to really tease out someone's chronotype or time of day preference is to ask them, "okay, if you could go to bed and get up with no distractions, no alarm or kids running in the room, if they could do that in their dream life, what time would you get up?" And that's your answer.

It's when they would go to bed and get up with no external time pressure. What's their sleep schedule? And so someone that would say, "God, I'd love to be in bed at nine, and I like to get up at six"... Boom! Then you've got your idea that you could assume from that their biological timing is going to be earlier in that evening and the clock time evening, someone says to you, ah, actually I like staying up till about one ish and I'd love to sleep till nine or 10. Then you know that again they're later. There's possibly a bit more time to work with in what we would call the clock time evening.

Danny Lennon: Yeah, and I certainly wouldn't let some of the intricacies of this paper derail us from not having any conclusions because first of all, even without knowing someone's genetic variant as we noted in, in some of these graphs, regardless of that, you still see differences based on that earlier or later feeding.

And also when we think of the impact between timing of meals and glucose response, that is not just down to melatonin or not just down to circadian phase. Those are two probably of the primary drivers, but there's also that behavioral cycle Alan outlined. And this is where we probably have most control of light exposure. What we tend to do when we're rest, when we're active, when we're going to have consumed meals. All of those are within this behavioral con. I would say control in most cases, but sometimes that is not the case, but at least a factor to consider that things can be done. So I think those are our three big things to look at the melatonin, the circadian phase, and then the actual behavioral cycle.

And then there can be some practicalities, like I said, around think you have that few hours before normal bedtime, and then how important that is then becomes more of a discussion of that individual's situation. So again, it's if someone has pre-diabetes, this is going to be far more relevant than if someone is doesn't have pre-diabetes, is very active and has just done a training session an hour or two before that, and they're going to be able to dispose of that glucose quite normally. So again, the implications of this, we don't need to go too far and say no one should ever eat within three hours of going to bed, ever. That is again, probably not what is being said.

Alan Flanagan: Yeah, I think so. And the, the individual level application of this is going to differ substantially based on those factors. And I think the, I guess the main real take home with these two cycles, we've got our circadian cycle and our behavioral cycle is the behavioral cycle is the largely modifiable part of this, right? The your endogenous circadian rhythms are, like I said, primarily genetic and then influenced by sex and age. And , they're not modifiable factors for the most part. So it's trying to get people insofar as they can to try and think about how those behavioral components, like their sleep wake timing and their meal timing aligns with that unmodifiable underlying biological stuff.