Danny Lennon: Dr. Alpana Shukla, thank you so much for taking the time out and joining me on the podcast today. You're very welcomed.

Alpana Shukla: Thank you very much Danny. I am delighted to discuss our Food Order Research with you and I look forward to discussing this in some detail, and to your questions.

Danny Lennon: Yes, I do have quite a few because I've really enjoyed looking at this area of work. I think it's quite fascinating and the big thing is that there's some I think important implications potentially of this down the line for those working with people, particularly in the areas of pre-diabetes and type-2 diabetes as we'll probably discuss. But before we get to the paper specifically maybe to start can you give people an idea of your own background of what led you to get started in this field and this are of research in particular and how that came about?

Alpana Shukla: I am an endocrinologist by training, and I've actually had a fairly global experience as far as my training is concerned. I graduated first from Grant Medical College in Mumbai, and then I went on I trained in the UK, which included my time in both England and Ireland. I also trained in Australia and now I'm in the US. And really at this stage of my career I've been involved in research for the past eight years while I've been with Weill Cornell Medical College. And the key areas of my interest are behavioral interventions for treatment of obesity and diabetes as well as pharmacotherapy for obesity and metabolic surgery. So, the reason why I actually got really in this particular behavioral

intervention is that as a physician who's spent a lot of time actually managing patients with diabetes, I recognized that while we always counseled our patients on reducing their carbohydrate intake, on the type of carbohydrates they should consume, and the quantity of carbohydrate they should consumer because these have historically been the key predictors of glucose response after a meal. I fully recognize though that it's often difficult for patients to actually comply with these recommendations because somehow we all do love carbs, and so is there a way to actually mitigate the effects of carbs. And I think that's where this whole intervention really fits in.

Danny Lennon: Yeah. I think that's a key point we'll probably circle back to later on that whilst there are certain things in ideal world we'd like to do we also need to have things that are helpful in practice for situations where we know compliance with diet is not going to be a 100% which is nearly all the time, I mean, trying to get people consistently to adhere to just some basic tenets can be difficult, never mind something they are restricting consistently. But just to start off, obviously you started looking at this particular area related to food order and we'll explore what that means in just a moment, and we'll touch on some of the recent research papers from maybe 2015 onwards you've published in this area. But why was it that you and your colleagues started wanting to look at this particular idea of the order we consumer nutrients or at least what was the hypothesis, and why mechanistically would it make sense that this could potentially play a role or help us to some degree?

Alpana Shukla: Right. So, the term Food Order refers to the, as I said, refers to the nutrient sequence during a meal, and specifically it refers to the order in which the carbohydrate component of the meal is actually consumed. And so, our hypothesis when we started off, Dr. Arrone and myself and our entire team here at the Comprehensive Weight Control Center at Weill Cornell Medical College, our hypothesis was that the timing of carbohydrate ingestion during a meal has a very significant impact on the postprandial glucose level.

And our working hypothesis was that when the carbohydrate component of the meal is consumed at the end of the meal or consumed last the effect would be much less compared to when it is consumed at the beginning of the meal. In other words, if you start the meal by having proteins and vegetables first and save the

carbs for the end we hypothesized that this would have a lower impact on the glucose levels.

Now having said that, when we started off the first study which was way back in 2015, it was the first pilot, so we were really looking to actually generate an idea and we believe that this would have an impact. There was some data in this field that had been published by a Japanese group, it suggested that if you had vegetables before you had rice compared to having the same meal in the reverse order glucose levels might look different. Our question was how would this actually translate in the context of a real meal, a complete meal. The kind of meal that you and I actually have every day, which has – it has vegetables, it has protein, it has carb and it actually represents the food that people eat in the real world, and that's where we actually started off.

Danny Lennon: Right. And so, like you said you had that pilot trial in 2015, and since then there's been some more trials looking at this. So, maybe as a best place to start off with some of the recent work that you think has really shed some light on answering this question. Can you maybe explain how you set up those trials, how they were conducted, some of the methodology behind them that you're going to do to try and test and explore this idea at least for some of the more recent studies at least?

Alpana Shukla: Sure. So, I think the best thing might be for me to just walk through the three studies because really I look at these three studies, we call it the Food Order Trilogy in our group, and they've really served as thesis projects for a lot of my students who've been doing their Masters in Nutrition, and so it's been a wonderful experience for all of us working on these three studies.

So, when we started the first study we basically had 11 patients, it was a pilot study, and we had 11 patients with well controlled type-2 diabetes who were taking metformin and what we did was we had them come into our Clinical and Translational Science Center on two separate days, a week apart, and we had them consume the exact same meal in two different ways. So, I want to emphasize that the meal was the same, so gram-for-gram and carb-for-carb it was the exact same meal. And it was made up of freshly baked ciabatta roll, orange juice which served as a carbohydrate component of the meal, and there was a salad, and then there was grilled chicken which was the protein component of the meal. The first time patients came in for the study they had

the cibatta roll and the orange juice first, and then they waited for 15 minutes, and then they had chicken and vegetables. They came back a week later and they had the exact same meal. The only thing that they did different was the order, so they had the protein and vegetables first and they had the bread and the orange juice at the end of the meal. And we sampled their blood for glucose and insulin at 30-minute intervals, so that was the first study.

The second study we – the results of this first study were so dramatic that we felt that just really as very responsible researchers we needed to actually validate these results ourselves, and repeat this, and confirm that these were really true. And so, we redid the study but with a slightly bigger sample size of 16 patients and this time we also introduced a third intervention which was having patients eat all meal components together as a sandwich, because we recognized that in the real world that's often the commonest way in which people eat which is actually eat everything together. And so, in the second study in addition to actually sampling glucose and insulin which we did the first time we also wanted to understand the mechanisms, the hormonal mechanisms that might underlie these changes we were seeing. And also, we also studied GLP-1, glucagon as well as ghrelin responses in addition to glucose and insulin. So, that was the second study, and then we wanted to take this further into a group which we felt where we potentially had a chance to actually impact much bigger population which is the pre-diabetic population. And as you know Danny yourself, this is a huge group which is actually growing worldwide, and in fact in the US currently about 38% of the US adult population has pre-diabetes and obviously is at risk of developing diabetes if it's not checked.

And so, our third study we sought to actually asses how these results would pan out in this particular population, and also we wanted to assess whether we would get the same kind of results if we changed the macronutrient composition of the meal, so we changed the macronutrient composition for the third study. And in the third study we also had a separate intervention which was in addition to having patients eat the carbs and the carbs last third group actually had an intervention where they had only the vegetables along with a olive oil dressing which was followed by the protein and carbs. And the reason we did this intervention was because we recognized that for a lot of cultural group it didn't quite resonate very well for them to have both the proteins and

vegetables first, and have carbohydrates separately at the end. And so, our question really was also to see if some of those benefits or most of the benefits of food order could actually be engineered by just simply having patients eat a big salad with the dressing initially and then have the rest of the meal, which might actually be easier for lot of people to do.

Danny Lennon: Yeah. I really love the way you brought us through each of those things because you can see the evolution of being able to ask why are some of these things happening. And I going from that pilot we notice a certain result, and then thinking well what other interventions can we test that would give us some answers about the real world. And for each of those I definitely want to dig into some of the details and I am sure at this point people are probably assuming that there was clear differences in the results of each of those trials the fact that there was a second and third trial. So, with the first one just to recap for people that was looking at this meal of ciabatta and orange juice with salad and grilled chicken, and then either those carbohydrate dense foods at the start or the end. And in that you were looking at the glucose and insulin response only. You mentioned that was at 30-minute intervals. How long after the meal did you continue monitoring those, and then maybe can you just actually bring us through what exactly you saw results wise that stood out the most in that particular trial?

Alpana Shukla: Yes. So, in the first study the time zero was the time at which the – actually just before the start of the meal, and so the 30-minute time point was 30 minutes after the start of the meal. And then we sampled for every 30 minutes until 120 minutes. So, the other important difference I had to point out between the first study and the subsequent two studies we did was also the duration of follow up. So, the first one was 120 minutes, and then subsequently we did it for up to 180 minutes. And as far as the results were concerned in the first study what we found was that the incremental area the curve for glucose was about 73% lower and for insulin was about 48% lower when the vegetables and proteins were consumed first before the carbohydrate compared to the reverse food order when the carbohydrates were consumed first. We actually had the table of the actual results, but the graph actually isn't the paper which I regret deeply but it is in our office. We actually show this graph to all our patients as part of the nutrition counseling we offer them. In fact, I have to say this that this graph works quickly through a 1,000 words

because we show this graph to patients, it stays with them and they could see how dramatic the effect of just a simple intervention could be.

Danny Lennon: Yeah, and that's the thing. It's like those are extremely dramatic results. They are not these small little negligible changes. They are dramatic in nature, and you say from not changing your food intake just simply changing the order. I really like that for the second trial then you've acknowledged okay well maybe for certain meals it's going to be impossible for people to have their protein and fibrous vegetables first, and then wait awhile and have carbohydrates not always easy to separate them out. For example, like in a sandwich or maybe it's a mixed dish. So, you looked at the mixed meal in the second trial in addition to the carbohydrate last and carbohydrate first I believe. So, with that what did you see with that mixed meal, and where do that compare compared to the carbohydrate last and carbohydrate first conditions?

Alpana Shukla: What we found was that the mixed meal was in between the two food orders. So, the results were intermediate between eating the carbs first and eating the carbs last, but it was closer to the carbs first compared to the carbs last. And I think the reason – because we were hoping it would sort of sit somewhere exactly in between but it wasn't quite like that, and I think the reason for that was that we had patients take orange juice and I think the impact of taking a very – consuming carbohydrate that's very quickly absorbed is so powerful that even if you are having proteins and vegetables at the same time it doesn't dampen your sugar as much as you would like it to.

Danny Lennon: Within that study you also mentioned you looked at GLP-1, and glucagon, and potentially some others. What were the results you saw on that end and what might those results implicate?

Alpana Shukla: Right. So, I think what was very interesting was that we found that the GLP-1 response when patients consumed the carbohydrate portion last was greater than when they actually consumed the carbohydrate portion first, and this is actually very interesting because we saw a higher GLP-1 response but a lower insulin response when patients actually had the carbohydrate portion last compared to carbohydrate portion first. So, I think this is actually a very unusual response, and I think particularly because when you look at this particular response and compared to some

of the other interventions that people have studied before things like protein pre-loads. I think that's the sort of study that you might actually compare our intervention to, which is you know it's been studied by several investigators giving whey protein prior to a meal and that has also been show to actually lower the glucose level post meal. But that actually occurs with the insulin response being much higher and the GLP-1 response being higher. But with the food order intervention what we see is that the GLP-1 response was higher, but the insulin response was actually lower and I think that in a sense we think this is actually a good thing, because it means that if by eating in a certain way you are actually requiring less insulin to dispose the same amount of carbohydrate then you are kind of preserving your beta-cell function better. This is what we believe would be the practical implication of that intervention. Obviously, this is something that we need to study prospectively and test it out, but that's how we think this is what it means.

The other thing that we found which is very interesting because like I said, the two focus areas of our research are diabetes and the other area that we're very interested in is also weight management. And like you know and type-2 diabetes are inextricably linked, and so obviously the question that we wanted to look at is would this intervention have any impact on satiety and perhaps help in weight management. And to that end we think that some of the results we got from the hormones that we tested suggest that that may be the case because as you know the GLP-1 is also – it's a hormone that does signal fullness and satiety, so that might be one mechanism.

The second thing is that we looked at ghrelin levels, and I think these results we actually published separately in Diabetes Care as well just looking at the ghrelin suppression following the three food order interventions that is having the carbs first, carbs last or eating everything together as a sandwich and what we found was that when patients actually had the carbs at the end of the meal the ghrelin levels were suppressed for longer. So, at the end of 180 minutes ghrelin levels remained suppressed, whereas when patients actually ate the carbs first we saw an initial suppression of ghrelin which is what you would expect but by the end of 180 minutes the ghrelin levels had rebounded to above the baseline. And so, I think that this may also have some implications for satiety and obviously something that needs to be tested

prospectively in the real world and that's one thing that we do plan to take forward now.

Danny Lennon: Yeah. So, much interesting stuff from there, especially when you mentioned that you had the increased GLP-1 but with a lower level of insulin and I am glad you brought up the protein loading research because that can be particularly interesting where they pre-load with typically whey protein like you mentioned before the meal. And obviously the higher insulin levels that occur with that makes sense because we know that by the amino acid profile of whey protein lot of them are quite insulinogenic, but it's cool that we now have these potential other strategies where you can get that enhancement of things that GLP-1 and suppression of ghrelin but without that insulin elevation. So, with that I was also interested to ask between the first study, and then study two and three you had a longer time course. You had the 180 minutes as opposed to 120. Did those differences that we saw with 120 minutes in the first study did that just persist even after the 180 minutes as well or did the longer timeframe see things even out a bit more or was it just as pronounced with that longer timeframe?

Alpana Shukla: I think that's a great question Danny. Yes, so I think with the longer follow-up of 180 minutes we saw that the differences were slightly less than they were with just the 120-minute follow-up and it was also the reason why we felt we had to do the study again and really look at longer term follow-up because typically after three hours people actually snack and eat something again, and so what you're going to see is just an overlapping curves in the real world. But at 180 minutes we also saw very significant changes, so while the first time the difference was 73%. The second time it was more in the range of 55%, but I think still very, very significant.

Danny Lennon: For sure. That's really interesting. The third study in particular I find it really cool because first of all you mentioned this was looking at people with pre-diabetes which obviously when we're looking at things like glycemic control and those glycemic excursions after a meal there were a group who you really want to pay attention to. One thing then that kind of relates to what we were just talking about with the protein pre-load was that while that can have some of the benefits that other researches has shown and causing that greater satiety, and so and changes in meals afterwards. What's different there is you're just essentially having something different to the meal itself, right? Just a

supplement that's a separate thing to the actual meal, whereas here in this trial you tried to take what the meal was going to look like anyway and just have the pre-load as the fiber or the salad portion of the meal. So, I think you said it was the fiber or salad vegetables with some olive oil, and then followed by the protein and carbohydrate. So, I think that's really interesting to see those parallels with something like the protein pre-load, but except for just taking that meal component. I know you also mentioned that there is a change in the macronutrient composition in the third trial. Can you just expand on that as well?

Alpana Shukla: Sure. So, in the first two studies we actually used very little fat in the meal. So, it was basically a very high protein content, and the fat content of the meal was actually of the range of about 11% to 12% which is really low in terms of fat content. But when people often ask me why did we choose this kind of meal, we actually simply base the meal of a typical sandwich that people actually in New York do order out from one of the chains called Lettie's chicken sandwich and we said let's look at a meal that people actually eat that's what we want to do. And so that's where it was, but actually doing the third study I said we really want to be certain that these effects are not only because we just gave so much protein and we didn't give as much fat and what is it really because of? And so the third time we did this we actually went with a macronutrient distribution which was approximately in the range of 40% carbs and 30% fat and 30% protein which closer to the RDAs that most societies would recommend, and probably more can be generalized to more meal patterns across the world. And the results again we were very happy and thrilled to see that even with the change in the macronutrient composition the results were very robust.

Danny Lennon: Yeah, and I think that's an important thing right with such a big difference where we're now looking at something that's 30% fat as opposed to close to the 10% you are still seeing those differences which is really cool considering that we know for example, fat is going to slow down digestion times and other things that we're still seeing those big differences in glycemic response which is cool, particularly in these pre-diabetic population. One another thing that I did want to ask and I know that you've already half answered this already is you mentioned that protein pre-load that other researchers looked at before like giving some whey protein before a meal, whereas here you looked at giving that kind of fiver and salad with some olive oil

before the actual protein and carb meal afterwards. How does that kind of meal pattern compare to some of the protein pre-load strategies both one at least mechanistically, and then two pragmatically in the real world if we were thinking about either of these strategies for recommending to a patient or a client?

Alpana Shukla: Right. Sure. So, mechanistically I think the key difference that you would be – the fact that when we do give a protein pre-load what you are doing is you are getting a higher insulin GLP-1 and higher insulin response. I think that's what's been shown repeatedly with several studies. Whereas with the food order intervention of having the vegetables and you know either just the salad with the olive oil dressing or the salad with the protein first what we have shown across the three studies really is that this intervention actually mechanistically what it does is it stimulates the higher GLP-1 response but it actually lowers your insulin response. So, I believe that an intervention that's reducing the amount of insulin that needs to be released after a meal sparing the beta-cell, it's protecting the beta-cell from developing full blown diabetes, and I think that in that sense this has some added merit. I think just in terms of practical application I believe that it was easier to tell patients to – that tell patients that yes you can have some carbs, you can have them at the end of the meal rather than say don't have carbs, so eat very little carbs which I think in the real world people find difficult to comply with. As to asking patients to take whey protein before a meal I think that's a kind of a advice I think some people can follow and I think typically the kind of patients I've seen in the real world who like the idea of doing whey protein, people who are really into gymming and you know in that space. But I think practical intervention for people who have diabetes are really thinking or have to be thinking about their glucose on a continuous basis. It's not like just when you're going to exercise it's really every single day all of the time. And I think in that context actually doing an intervention that simply means switching the order rather than actually eating something different might actually fit in better.

Danny Lennon: Yeah, for sure. And I think on the practical side as I think you mentioned towards the outset of the podcast is that in an ideal world sure there are some things that you might like to do with a patient. And I don't think it's too controversial to say for a type-2 diabetic population for example, that we want to have some control over the glycemic load of a meal because we want to look at what their blood glucose is going to do and it definitely can be

useful in the management of that disorder, especially when we consider if we get their calories appropriate, and their fiber and protein intake, and their fat intake all on point there's probably not going to be a huge amount left for large carbohydrate loads anyway. But of course as we mentioned compliance with diet is almost never 100% in the real world. So, I'm just wondering with this intervention do you see carbohydrate last food pattern as rather than a primary recommendation it could be also seen as a plan B option or essentially by that I mean ideally we have something we would like to do but in the case where someone is going to have a meal of a high glycemic load or higher than we may have planned for them then at least they have this contingency for okay for those cases I guess you'll have that meal, but I'm going to make this meal pattern changes to mitigate the glucose excursions at the time of that strategy? If that question make sense?

Alpana Shukla: Yes, absolutely Danny. I think it makes perfect sense. I think although we did in this experiment as we call it we controlled everything very perfectly. So, I had my students actually sit in with the patients when they were in the Clinical and Translational Science Center, and so we ensured that everybody ate everything each time, so that there were not confounders. But I think that in the real world when people actually eat in this order. When they actually have proteins and vegetables first, they start a meal like this they're actually going to end up eating less carbs. I think that's our practical experience of actually – I use this intervention. But I think what's really interesting about what we found in these three studies is that even when people eat the same meal and they eat everything even so the glucose is much lower. But I think in the real world the effects would actually be even better.

Danny Lennon: Yeah, for sure. I think that's extremely plausible thing for sure. One another thing I did want to ask is whilst this work has clearly been focused on pre-diabetes and diabetes, and therefore glycemic control, and I'll ask about some future plans in that direction in a moment. But are the any plans or any hypotheses that you guys have been thinking about to examine the impact of a food order or this carbohydrate meal pattern and other impacts relevant to health. For example, like blood lipids or like free fatty acid levels after the meals or even people's daily caloric intake that it's going to have, is there any plans or hypotheses of how it may influence other health markers outside of glycemic control and some of those satiety hormones?

Alpana Shukla: Yes, absolutely. I think we have been talking about this and certainly looking at the effects of this intervention on lipids and free fatty acids is definitely along in the pipeline. The other area we want to also look at is just looking at this intervention in people who have either overweight or obesity and are normal glycemic have no diabetes or pre-diabetes, and then look at the gut hormones and see if those look different with this intervention because we did show that it has effects on ghrelin as well as GLP-1 in patients who have diabetes but we haven't studied this in the normal population and that is definitely one of our future projects.

Danny Lennon: Awesome. And so, just keeping in line with that in relation to some of these studies that came out what is the kind of next steps in this area of research? What are the next big questions you hope to answer or what kind of work kind of work is underway at the moment that we may see some work come out over the next year or two?

Alpana Shukla: So, right now the next step for us really is to assess the practical feasibility of doing this in the real world. So, it looks beautiful in the experimental setting, but in the real world can people do this? And if they do this, is it going to impact their glucose levels? And so, in this way we've actually set up two prospective studies and I am very happy to share this information with you. The first one is in women with gestational diabetes, and this is actually a feasibility study and we felt like this might be a great group to assess feasibility because this is the time when we find women are extremely compliant with behavioral interventions and any intervention that might potentially have a favorable impact on their outcome as well as fetal outcome is likely to be followed. And so, that's our first group in which we're actually assessing the practical feasibility of this intervention and that study is ongoing right now.

The second group that we are testing this intervention in, and this is a pilot study just looking at both feasibility as well as effectiveness, is in patients with pre-diabetes. We've received funding for this and we are going to start enrolling patients possibly by the end of this year. We're looking at both glycemic as well as weight as outcomes in that study. So, those are the two areas in which we're taking this research forward and of course

we have some of those mechanistic studies still to be done in patients who do not have diabetes or pre-diabetes as well.

Danny Lennon: Yeah. It's so interesting and I can't wait to see more of this work emerge over the coming years, because as you say, there's lots of explore between looking at the mechanisms as well as looking at bigger picture overview of not only I suppose the glycemic response to certain meals but what might do to someone's like 24-hour glucose Hemoglobin A1c or something like that over a longer term is going to be really interesting to see. So, I definitely look forward to hearing about more of your work Dr. Shukla. Before I let you go and before I get to the very final question for people listening who are interested to learn more about this or to keep up-to-date with some of your work or your publications online are there any best places that you'd advice them to go like a ResearchGate profile or Twitter or anything like that where they might be able to keep up-to-date with some of the work that you're publishing?

Alpana Shukla: Right. I guess the easiest way to this is simply on PubMed. All of our publications are on PubMed. Unfortunately, I am not very Twitter savvy. I do have a Twitter account, but I probably should work more on that. But at this point you know seven of our papers have been actually covered extensively by several media as well as other news outlets, and so I think it's quite easy to find them but everything can be found on PubMed.

Danny Lennon: Yeah. And I think you're probably doing the right thing by staying away from Twitter. I think a lot of people would be a lot more productive if that was the case, so I definitely don't think that's a downside. And for everyone listening I will link up to all the studies that we've talked about today in the show notes to this episode, so you can go and click through and read those which I encourage that you do. Really is fascinating for those who are into reading research. And so, with that Dr. Shukla that brings me to the final question I always finish the show on, and it can be to do with anything even outside of today's topic and it's simply if you could advice people to do one thing each day that would have a positive benefit on any area of their life what would that one thing be?

Alpana Shukla: Thank you Danny. And I want to share something that I actually tell myself every day and I also tell my students which is find your

passion and pursue it relentlessly. It's not a question of 'if' but how you can do it.

Danny Lennon: Brilliant. A great way to finish this and with that Dr. Shukla I want to say thank you number one for giving up your time to do this. I've really, really enjoyed this conversation, but beyond that I've really enjoyed reading your work and know that it's very much appreciated. So, thank you for what you've done and thank you for the great information that you have given throughout the course of this discussion today.

Alpana Shukla: Thank you Danny. It was a pleasure talking to you.