#436_ Charlene Van Buiten, PhD – Coeliac Disease & the Search for Novel Therapies PUBLIC

DANNY LENNON: Here we are, a big welcome to the podcast to Dr. Charlene Van Buiten, how are you doing today?

CHARLENE VAN BUITEN: I'm doing well. Thanks so much for having me.

DANNY LENNON: I'm excited to talk about some of your work because as I think I mentioned to you before we started recording, it's very novel and could be potentially very informative and important going forward, but before we get into any of that, maybe to lay the groundwork before we talk about anything else, can you maybe just give people a bit of context to who you are, and what your work typically looks like, where you're based, anything else that will be of relevance for the rest of our conversation?

CHARLENE VAN BUITEN: Yeah, absolutely. So I am a third year assistant professor at Colorado State University in Fort Collins, Colorado, and I'm a member of our Department of Food Science and Human Nutrition. My work specifically is more in the food science end of this. So my training, actually I have a bachelor's in Nutritional Science from the University of Connecticut, went on to do a PhD in Food Science at Pennsylvania State University, and then a two-year postdoc in the Department of Plant

Biology at Rutgers University, by way of the NIH, National Center for Complementary and Integrative Health, studying plant bioactives through that postdoc. So I've been here since 2019, I direct the food structure and function lab, and we're interested in interactions between nutrients in plant based foods, and how those interactions can affect human health.

DANNY LENNON: Fantastic. And, of course, one large part that we're going to focus on today is various disorders of the gastrointestinal tract, of which we'll probably mention a few, but, in particular, you've focus a lot of your work looking at celiac disease. So maybe as a good starting point, maybe one of the first few things that you would want people to know about celiac disease generally, of course, most people have heard of that, but what are the important things in terms of why this is such a big issue to look for future interventions, and then, maybe an overview of how we would typically classify that what are some things we should know about diagnosing it, and then what actually happens with someone that has celiac disease?

CHARLENE VAN BUITEN: Sure. So celiac disease is a fascinating disorder, it affects approximately 1% of the global population, which is quite a lot of people, and despite that and its increase in prevalence, we actually don't have a cure for celiac disease beyond strict lifelong adherence to a gluten free diet. So celiac disease is typically thought of sort of in the same terms as a food allergy, but it's actually not, it's an intolerance, so it's sort of a different immune mechanism. And with individuals who have celiac disease, what happens is when they consume gluten protein, that protein is broken down in their gut, and then it stimulates a cascade of inflammatory and immune responses. And part of those immune responses, we first have an innate response, which is associated with just general inflammation of the gastrointestinal tract, but then, we also have an adaptive response where the body is actually mounting an attack against

not only gluten proteins, but also enzymes that are produced by this damaged gut lining. So that's really where the auto immune portion of celiac disease comes in, and so, this is really important to sort of look at treatments for this because, as I mentioned, it affects a lot of people, and it's increasing in prevalence as well. And on top of that, it's stimulated by the ingestion of gluten protein, which we find in wheat, barley and rye, and obviously, products made from flours from wheat, barley, and rye. But we also see it popping up as a processing agent, a thickener, so a lot of other just plain ingredients in food product. So we see it showing up in products where you wouldn't typically expect it.

DANNY LENNON: Fantastic. One of the detailed aspects that may crop its head later in our conversation comes about when we start looking at the kind of different subunits of what we typically call gluten, and we look at these different subunits, and even beyond that, some of the structure and the amino acids, can you maybe just discuss some of the ways we might break that down and the significance of that, particularly, for this conversation going forward?

CHARLENE VAN BUITEN: Sure. So gluten is a heterogeneous protein, which means that it's a class of proteins, rather than just a single protein that we can just isolate the way we can with others. And in that class of proteins, we have two subunits, so we have glutenins and we have gliadins. Glutenins are kind of more fibrous, they have a greater number of cysteine residues, they contribute that elastic property to a dough, for example. And gliadins, on the other hand, are really rich in glutamine and proline residues, and those will form more of a viscous texture when isolated. But when we mix these together, we get gluten, which is viscoelastic, and it's what contributes sort of that springiness to bread, for example, stretchiness to bread and pasta dough. So from a functional perspective, these amino acids allow for this structure to form that's really desirable in food products, but it's

also these specific amino acids that form the epitopes that are recognized by individuals with celiac disease.

DANNY LENNON: Thanks, yeah, and like we said, we'll probably circle back to some of that later on. I'm also trying to think of some other aspects that might be useful to classify that people maybe have heard about, but aren't really exactly sure on what that is, and I think one of the big things that gets discussed in this area with gluten generally is this loosening of those kind of tight junctures between some of the gut lining and, in particular, one of the things that gets implicated in that is Zonulin. Can you maybe just describe what Zonulin is and then its relationship to gluten more broadly, and how you would get people to envision this concept?

CHARLENE VAN BUITEN: Yeah, absolutely. So kind of one of the hallmarks of celiac disease pathogenesis is this initial loosening of the gut barrier, so if you imagine your gut barrier just as a line of individual epithelial cells, they're all kind of stuck together with these proteins called tight junction protein. And these tight junction proteins can release, so we can downregulate them, we can upregulate them, and sort of affect the permeability of the gut barrier. And there are a few different diseases that we see increased permeability in, so not just celiac disease, we see increased gut barrier permeability and inflammatory bowel disease, and we can see this also associated with, for example, obesity and metabolic syndrome. We can see it in chronic kidney disease as well. So we do see barrier permeability in a lot of these increased inflammatory states, but what is fairly unique about barrier permeability in celiac disease is that when an individual consumes gluten, this gluten is able to bind to a receptor on intestinal epithelial cells called the CXCR3 receptor. And that receptor stimulates the release of Zonulin, this molecular messenger that will essentially tell the cell to disrupt tight junction protein. Interestingly, this Zonulin trigger is actually what we see

produced in response to cholera toxin as well, so we're following kind of this same mechanism as a cholera infection, which is crazy.

DANNY LENNON: Fascinating. And again, this is probably something we will circle back to in the context of this conversation, now, one of the things that is interesting about your work as you've already noted, right now, in terms of actual treatments, what people are just actually left with is a restrictive diet where they have to avoid gluten altogether lifelong. So it's obviously a very interesting area to see, are there other ways that people can be helped, and one of the areas that your work in particular has been at the forefront of has been hypothesizing this potential role for plant bioactives, specifically, polyphenols. So can you maybe outline where that line of thinking or that hypothesis comes from, in other words, why polyphenols, why is it something that we might be looking at in the context of celiac disease?

CHARLENE VAN BUITEN: Yeah, absolutely, so if we look at sort of the last, say, 20 years of attempts at finding a treatment for a celiac disease, you can kind of break these treatments into two different categories, we have treatments that target gluten protein, so they're either trying to break it down to the point that it's unrecognizable, or trying to bind it so that it just passes through the body without being absorbed at all. So we have these gluten focused therapies, or we have these sort of patient focused therapies. So, for example, the introduction of immunomodulators, so trying to dampen this overactive immune system, something that will affect the individual directly, so if we sort of look at those two treatments in a silo, I think for most people, we would say, well, what if we could do both, what if we could potentially modify the immune system at the same time as preventing the digestion, for example, of gluten, and that's where the idea to use polyphenols really came from. So polyphenols have been studied extensively for their ability

to reduce inflammation, for their ability to potentially modify immune signaling in that way, but they've also been studied extensively, especially in food science, for their ability to interact with proteins. So one example of protein polyphenol interactions that I think most people are pretty familiar with is actually when you take a sip of red wine, the polyphenols, the tannins in that red wine will bind to your salivary proteins on your tongue, and that's what gives you that oral sensation of astringency. So I actually came from a research lab where we were studying wine at the time, and I learned about this phenomenon of astringency, but I was also interested in celiac disease and in gluten proteins and realized that gluten is really rich in proline, which is similar to salivary proteins. So I thought, okay, well if we know that our salivary proteins interact with polyphenols, does gluten interact with polyphenols, and how can that potentially affect digestion. So that's really where my line of work started, and kind of, as we've moved forward with this project, we're really interested in identifying how different structures of polyphenols can affect their specific role within the pathogenesis of celiac disease. So we have a wide array of polyphenols from a really diverse, really diverse structural characteristics, so some might be better at binding gluten proteins, some might be better at eliciting this immunomodulatory anti-inflammatory response. So what we're trying to do right now is kind of figure out the optimal mixture of those and how we can create sort of the optimal nutraceutical treatment here.

DANNY LENNON: Fantastic. So there's some sort of potential interaction between certain polyphenols, and these proline residues, which you've noticed is part of gluten, and so, with that interaction, something could be going on there. Is there anything else, especially for people who want to maybe dig into the details mechanistically of what's going on with some of these protein polyphenol interactions, is there anything else that you would make them aware of at this

point that might be useful to frame some of this?

CHARLENE VAN BUITEN: Sure. Well, I think, first and foremost, it should be noted that all of the work that's been done to this point is in vitro, so we are really looking forward to getting more involved with some animal studies, and potentially some clinical trials. Now that I am a professor with my own lab, we can really pursue those things funding dependent, so that's really important to note, and I think also, for people who are really interested in this topic, it is important to note that we don't at this time know the sort of best way to apply this concept. So whether it's something that needs to be a co-treatment or a pre-treatment, so something you take before you go out to dinner, would it be enough to take the place of a gluten free diet, we're not entirely sure at this time. But our studies have shown that green tea extract is able to prevent gluten stimulated inflammation in vitro.

DANNY LENNON: Super interesting. So you've noted there, in relation to some of those potential mechanisms that got you interested, that there's not only this anti-inflammatory effect of these polyphenols, but then also their ability to interact with that protein that could degrade it, or at least neutralize it in some degree of a way, or at least that's a potential way to look at it. And, of course, one of the things that I noted that you reference in some of the review papers that you've written is that we do see polyphenols looked at in other areas outside of celiac disease, for example, with ulcerative colitis and Crohn's. What do we see in those areas that may be applicable here, or at least can maybe lend some hypotheses that you would investigate in the future?

CHARLENE VAN BUITEN: The work with ulcerative colitis and Crohn's disease, I think has been a really good guide for this type of work, because those studies do often look at, for example, the mediation of intestinal permeability, the mediation of inflammation, due to just direct treatment with

polyphenols. So that's really provided a good foundation for us. I think there's also a lot of really interesting work being done on the influence of polyphenol supplementation on the gut microbiome, which I think could be a really interesting place to look for us as well.

DANNY LENNON: So if we maybe get into some more of the details of the work that you've done, you've already noted that there's a number of these in vitro trials, first, maybe from an overview level, before we get into anything else, can you maybe give us some insight into how those studies were set up, the kind of goal of those particular in vitro trials, and then, some of the findings that you thought were most interesting?

CHARLENE VAN BUITEN: Yeah, absolutely. So, our first study, which was really focused on the proof of concept, we wanted to investigate interactions between green tea extract and gluten protein. So for these studies, we focused on characterizing whether those interactions even happened. So the formation of colloidal aggregates was characterized in intestinal type conditions, so at a neutral, slightly acidic pH associated with the pH of the duodenum, so the small intestine where most of these interactions are going to take place, so really the site of celiac disease is going to be the small intestine. So we found that there were aggregates formed between green tea polyphenols and gluten, we also studied the effect of green tea extract addition on the digestion of gluten proteins. So what we did was combine our gluten protein with green tea extract, and then run this through an in vitro digestion, so we exposed these mixtures to pepsin, to trypsin with the associated pH drop, and increase again, and then we used an SDS page, which is a method for separating proteins by molecular weight, to assess the extent of digestion that was able to be achieved in the presence or absence of green tea extract. And what we found from this was that there was a dose dependent effect of green tea extract, and it was able to actually prevent the breakdown of gluten protein. And so, this is

important because the breakdown of gluten protein in the gut is a critical first step to that protein being recognized by the body as sort of this foreign invader. So by preventing the breakdown of it, we are essentially creating these protein polyphenol aggregates that we believe, and we will be testing whether these are able to just pass through to the feces, rather than being fully digested by the body. So once we found this, we were really interested in sort of figuring out what the mechanism for the prevention of digestion was, so was it going to be a direct interaction between proteins and polyphenols, or was it going to be kind of this anti-nutritional effect of our polyphenols from green tea extract, directly inhibiting our digestive enzymes of pepsin and trypsin. And we found that it actually was both, so green tea extract was able to inhibit pepsin, and was able to inhibit trypsin, so we think that's kind of this mixed mechanism here. So once we knew that we're seeing sort of this anti-nutritional prevention of digestion approach, we decided to test whether these protein polyphenol aggregates would be able to stimulate inflammation in the intestinal cells in vitro, the same way that gluten alone would be able to. So to do that, we use a model called the Caco-2 transwell model. Caco-2 cells are intestinal cells that will grow to form a confluent monolayer that's similar to the small intestinal barrier. So they'll express the same brush border enzymes, they express tight junction proteins, and it's a pretty simple but robust model of the small intestine. So we grew these transwells and we treated the apical layer of these transwells with our protein polyphenol complexes, and we had controls of polyphenols only as well as gluten only. And we found that when we had our green tea polyphenols treated, whether they were alone or in the presence of gluten, we saw an improvement in stabilization of barrier integrity, compared to when we treated these cells with just gluten, and we saw a disruption of that intestinal barrier and increase in permeability, and we saw associated changes in inflammatory

markers as well. So in the presence of gluten only, we saw increases in Interleukin 6 and Interleukin 8, IL-6 and IL-8, which are associated with barrier disruption. And we did not see increases in those markers in the presence of green tea extract.

DANNY LENNON: So given all this, and, of course, very, very interesting findings that could have big implications, but as you've already noted earlier, the caution around this is that this is, of course, in vitro work and you want to now replicate that in maybe animal models, and ultimately in human interventions is what we're ideally going for. Has there been previous cases in the past of things that have been looked at in the context of celiac disease, or maybe something similar, where there was initial, maybe in vitro promise or maybe even mechanistic work that was very promising, but then ultimately didn't play out, or are we still at the kind of early stages of looking at any interventions beyond a gluten free diet?

CHARLENE VAN BUITEN: So with celiac disease, it's been definitely a bumpy road in terms of finding treatments. Obviously, we don't have one other than the gluten free diet yet. There's definitely been some indicators of success for some treatments, there's a vaccine that went to phase 2 clinical trials, but ultimately it was pulled for being ineffective, so it had been shown to be effective up to that point, and then didn't work out, and there are several other treatments that are, I believe, currently still in phase 2 clinical trials as well. But it definitely is a caution that I think people always need to take when it comes to considering moving from in vitro work to in vivo. You see it all the time, especially in polyphenol research as well, you'll get these really incredible results of something being anti-inflammatory or antioxidant when you're just testing directly on cells by introducing these concepts to a much sort of messier model like mice or humans, you're introducing all of these other confounding variables, so what that person ate that day, and their gut microbiome,

and all of these other things that you really can't take into account for in vitro studies, so it's definitely important to always keep that in mind I think. But we're really looking forward to kind of moving towards that more complex matrix and seeing if this does hold up.

DANNY LENNON: Maybe let me ask you something in relation to your work personally, in the fact that you now have a lab established, you have this work that was really interesting and promising, and I'm presuming that you're very eager then to kind of build on that. But, of course, the past few years, the world has thrown us a pandemic in the works, and how has that been in relation to trying to get anything done really, but especially when you are trying to build on work that's there, and you have the establishment of a lab,, etc.?

CHARLENE VAN BUITEN: Yeah, it's definitely been a little slower going than I would have hoped for sure. So I started here in August 2019, so we shut down six or seven months after I began working as an assistant professor. So my lab setup has taken a little bit longer than I would have hoped, and it definitely is tough to see scientific progress from other laboratories that were more established than you before, you know, they're able to move much faster. But I will say my department has been extremely supportive, and I've been really lucky to hire some excellent graduate students who share my passion and my motivation for this work. So I think we're really at a point where we're starting to chug along and generate data and apply for grants, so it's going really well despite a slow start.

DANNY LENNON: Fantastic. I'm delighted to hear that. So given the fact that there is now these next steps to be taken, in relation to anything that we've discussed so far in this particular concept, what are those next immediate steps of what is the next kind of set of data that we would need to apply, and then even beyond that, what

ultimately, would you or other independent groups need to look at going forward?

CHARLENE VAN BUITEN: One of the really big questions is going to be how can this actually be applied. So can we consider this in the context of someone who has a diet that's really rich in polyphenols, they're going to be potentially more protected than someone who doesn't, or is it going to really be a hardcore nutraceutical, take this dose of polyphenols before you go out for a meal. So I think we really need to try and answer those questions. One thing that I'm really interested in is sort of elucidating the specific mechanisms for individual polyphenols based on their structure. So with green tea extract, our primary catechin in green tea is this compound called epigallocatechin gallate or EGCG. And I've done quite a bit of work on EGCG still within the context of celiac disease, looking at its binding to specific epitopes in gliadin with interest towards epitope masking, structural change, that kind of thing. But amongst EGCG, within that green tea extract, we also have epicatechin, we have epigallocatechin, we have just catechin, you know, there are all of these forms of polyphenols that likely don't bind to protein in the same way, and then also have different, for example, bioavailability is so different potential to affect that inflammatory pathway. So I'm really interested in kind of sussing out those details of, if we give someone a mixed dose of polyphenols what can we expect from that mixed dose. I mean, of course, I've worked on green tea up to this point, but there are so many other polyphenols to be explored as well, so black tea will have a whole different profile of polyphenols; grapeseed extract, a whole different profile. So I think it's really important to kind of develop an understanding of the structure functional relationships with these compounds when it comes to celiac disease.

DANNY LENNON: We could probably speculate on a whole host of different things, but I'm sure one thing that maybe some people are thinking is, well, given

that we're unsure of whether this intervention will actually be shown to have efficacy in humans yet, but even if it does, will it be appropriate enough to completely mean that someone can get treatment without needing a gluten free diet, or it'd be part of an adjunct therapy, etc., they might be thinking, well, what about someone who maybe doesn't have something as serious as celiac disease, but might be classified as, let's say, with non-celiac gluten sensitivity. And, of course, then that throws up a whole difficult proposal of, because there's still disagreement of, well, how do we even classify that, diagnosis can become difficult, how do we judge whether there's going to be benefit or not, where it's much more clear cut if someone with celiac disease is benefiting or not. Has that been even a thought that has came to anyone's mind yet, or is that too far into the future to be considering kind of speculative questions like that?

CHARLENE VAN BUITEN: I would say it's both, it's definitely far in the future, but it's certainly something that has come up in a lot of conversations, a lot of meetings, because celiac disease affects 1% of the global population, I believe there is an estimate made by a very prominent scientist in this field that non-celiac gluten sensitivity, so any kind of just gluten, uncomfortable feelings from consuming gluten will say, effects between 7 and 8% of the global population was his estimate. So, I mean, that's a lot of people, and I think, when we think about this work, and really what our goal is, which, of course, is to improve human health always, we really do need to keep that in mind. But I think it's difficult right now, because a lot of this non-celiac gluten sensitivity, people will sort of tie it to fermentable oligosaccharides, for example, which is not gluten. So I think once we kind of suss out what potential mechanisms for non-celiac gluten sensitivity are, it might be a little easier to kind of see where this work with polyphenols fits in.

DANNY LENNON: Yeah, for sure, and, like we said, we could speculate on a bunch of things. Before I get to the final couple of questions around this thing out, many of our listeners are involved in some way in academia, many are nutrition science students right now, maybe food science students, or they're getting involved in the area maybe early stage career, but for those particularly who are getting involved in the early stages of an undergraduate degree or maybe a graduate degree, and are thinking of different areas to go in relation to other nutrition science or food science, you've mentioned some of the interesting work you've done and focusing on in these particular studies, we mentioned some in vitro work, can you maybe just outline for people who might enjoy that type of work, what type of advice would you give to people who are getting involved in nutrition science or food science as a student, and when they're thinking about their later studies, or what different areas of academia they might like, or if they'd like to go into academia, and they're considering, well, this might be an avenue I'd like to go, how do you think they should go through that thought process of what type of lab I'd like to work in, what type of area, what are some of the criteria you maybe have talked to some of your students about?

CHARLENE VAN BUITEN: Yeah, I think the most important thing is to really do some soul searching and identify what you really like, you know, what really sets your soul on fire when it comes to learning. And I think if you have a hard time narrowing it down, then food science might be for you. I was really drawn to food science because I liked to do a little bit of everything, so I really liked chemistry, I really liked biology, but it also ties in kind of some sociology at times, it ties in agriculture on a broader scale, it ties in engineering, it ties in nutrition. So if you see yourself as a jack of all trades, I think food science might be your home, and I say that from personal experience. So, for example, in my lab, we have a wet chemistry lab that is

really focused on the analytical portion of our work. So we have circular dichroism spectroscopy, we use UV-Vis, we have a GC-MS, so we're really equipped to do some hardcore analytical chemistry, but we also have a cell room where we do all of our cell culture, our molecular biology. So if you want to do it all, food science and nutrition I think is a great spot, you know, just kind of explore, see what you like to do, and as long as you like your topic, I think you'll be happy with your choice to go into grad school.

DANNY LENNON: Awesome. Before I get to the very final question, where can people find more about you and your work online, is there anywhere in terms of websites, social media, any type of stuff that you'd send people's attention?

CHARLENE VAN BUITEN: Yeah, so my group has a website, the foodstructureandfunctionlab.com. I'm also on Twitter, cbvanbuiten on Twitter, and we have a CSU website as well, but I think my website provides a little more detail.

DANNY LENNON: Awesome. I will link to those in the show notes for everyone listening, so you can go and check those things out. And so, with that, we come to the very final question I always end the podcast on, and this can be to do with anything, even outside of what we've discussed today. And so, apologies for the broad nature of this question and putting you on the spot, but it's simply, if you could advise people to do one thing each day that would have a positive impact on any area of their life, what might that one thing be?

CHARLENE VAN BUITEN: One thing each day that has a positive impact on your life, I think this is the hardest question you've asked.

DANNY LENNON: I did warn you, it's a broad, yeah, that is a tricky one.

CHARLENE VAN BUITEN: It sounds really basic, and maybe this is revealing some of my own bad habits, but I think writing a to-do list every day when I get

into work has significantly improved my life. So maybe for those of you who struggle with time management organization, that might help you as well. I know for me I have a million things going on, so when I sit down and write it down, it does make me feel better.

DANNY LENNON: Yeah, I am certainly in that boat as well, if I didn't have one, I feel all over the place, I can't settle until I have it done. So I certainly agree with that. With that, Dr. Van Buiten, thank you so much for taking the time to come and talk about your work today, thank you for this conversation. I've really, really enjoyed it, and I'm looking forward to seeing more work from you and your lab in the future.

CHARLENE VAN BUITEN: Thanks so much. Thanks for having me.

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