DANNY LENNON: Okay, so today, we are going to be talking about the general topic of how the gut microbiota may impact mood and mood related disorders, as we're going to discuss, and then, particularly focusing in on the potential role that some of you listening may have heard about for certain probiotic supplementation or psychedelics, as we may describe a bit later on. And this is a topic that I find incredibly interesting, and there's lots for us to explore here. On a kind of personal note, I remember when I was doing my masters initially that was in UCC, and so, for those who don't know, that was one of the places where, at the time, the gut microbiome lab was a huge deal there. Now the APC probably is still one of the leaders in the world of research in this particular area as we'll discuss, but that was also very much a big deal kind of around campus, if you're in nutritional science type area. A lot of it was actually mainstream news, like you'd see a lot of the big names that we'll probably discuss, like, getting onto like new segments, talking about some of their work. And a lot of that was kind of like based around some of what we'll discuss today, of how some of these changes may impact things like anxiety or mood related disorders, which was very much kind of groundbreaking at that time, but was very much in rodents. And

since that time, there's obviously been a ton of more work done that we're going to discuss today.

Actually, funnily, I have a kind of side note anecdote that you might enjoy. A close friend of mine, Ciaran O'Regan, who Alan you know, is particularly interested in this type of stuff, and he lives in Cork. But years back, after we'd been talking about some of the work that was being done there, and Professor John Cryan has been lead author on much of this work, he was on the podcast back in episode 105, if people want to check that out. But Ciaran spotted him out one weekend, walking around some market, he was there with his family and his kids, Ciaran was across the road, and almost he said, it was like, I was just shocked and star-struck because I've been reading so much of his research, and had seen him on these things I was looking into recently. So he just shouted across and pointed out and he goes, John Cryan, and he looked, and he goes, microbiome, and that's all he could think of saying. He was like stunned, and the family were looking at, was like, what is going on. He said, what happened. He's like... he kind of just turned and shuffled his kids away to safety and he left. And he was like, his family must think that's the weirdest thing ever, oh, like you're a celebrity walking around Cork City for your work on the microbiome.

ALAN FLANAGAN: Right, it's like, when does the scientist...

DANNY LENNON: Right, and especially there because we're looking at, to describe Ciaran for you, like, he's a big dude, but like, he was walking around probably in like Thai boxing shorts and going running around with like these long hair and looking like a maniac. And then John Cryan's wife looks at him shouting microbiome and nothing else.

ALAN FLANAGAN: That's amazing. That is brilliant.

DANNY LENNON: But that goes to serve into my idea of like, they were kind of a big deal around there and still

are, but very much remember, during my time doing my masters, a lot of the work they're getting discussed, which we're going to talk about today. And since then, obviously, books have been written, then there's other supplement companies have been all over this area because of that too. But we're going to try and dig into this question of, well, are interventions, either through diet or probiotic supplementation, actually able to have a real impact in either, say, treating mood disorders or otherwise. So we have published a statement on the website, Niamh, was the kind of lead research and author of this particular statement. If you haven't read that, go and check that out. But much of that will give some context for what we'll discuss today. So with that, maybe Niamh, I'll get you to open up here before we get into actually the interventions, and the psychobiotics specifically, we should probably start layering up some context for people listening. So first of all, gut brain access or the gut brain microbiota access, can you maybe define that, explain some of that for people, maybe get into some of the physiology that might be useful foundation for us?

NIAMH ASPELL: Yeah, sure, of course, I can. So, as you mentioned, it is kind of summarized in a little bit more detail on that statement online, so if anybody wants to go and have a little bit more of a look at detail, or look at some of those references, do check it out. But there's been a lot of work done in this area. So, kind of, when I was looking into the evidence, there's a lot of research that now we better understand the link between the brain and the gut, or this connection between the brain and the gut. So it's really well established, and it's been shown in regulation of lots of different things like appetite and mood. But there's a bidirectional exchange or this two-way exchange of information between our central nervous systems, essentially, your brain and your spinal cord, and then the system of the gut, your enteric nervous system on your GI tract. So they all work together to send information. So

there's afferent and efferent neurons, so sending information to and from the different parts of the nervous system, so they all communicate together. So in humans, the nervous system is, I suppose, divided into two divisions. So you've got your CNS, your central nervous system, and you've got your peripheral nervous system, which extends beyond the brain and the spinal cord. So everything that goes down your arms and your legs into your guts, so the peripheral nervous system is divided further into two main systems, autonomic and somatic. So for the gut brain interaction, it's focused more on this autonomic nervous system, and your enteric or your nervous system within your gut. So if you're probably familiar with some of the centers of the autonomic nervous system, so it's broken into two branches, the sympathetic, that's your fight or flight, and then your parasympathetic, so when you're kind of rest and recovery type systems. So the gut brain axis of the ANS or the autonomic nervous system sends information between the gut and the brain, so it's quite a complex system. But simply kind of if you have a meal, and the presence of food or nutrients, and your GI tract initiates or sends neural and hormonal responses to your brain, so your ANS system sends information from the cells in the wall of your gut, and it communicates this to the brain, and then you can then act and make decisions on what information is essentially returned. There is also, it's kind of important to note, because a lot of the studies we'll talk about later, kind of, look a lot more at the kind of immune modulating effect. So the gut also has its own immune system, and this communication pathway also integrates immune and endocrine systems as well. And the microbiota, this is seen as like a really important factor in how the gut brain access functions, and there's been a lot of research, a lot of studies showing that disturbances have been observed in many different conditions, particularly neurological and psychiatric

conditions as well. So there's a lot of evidence there.

DANNY LENNON: Before we may be turn to that, one of the things that you did note in that statement is that whilst there's probably still some quite open questions about this, there's been a number of different mechanisms proposed by which the microbiota are having this effect on neurophysiology more broadly. Can you maybe just touch on those, because I think they might be important?

NIAMH ASPELL: Yeah, there's been lots there. So in this area of research, there's a lot of animal studies. So most of the research is kind of preclinical research. The main systems that have been investigated is the hypothalamus pituitary adrenal axis, so the HPA axis. So this secretes hormones from your nervous and endocrine system, so if you're under stress, or if you're in a stressful situation, it promotes the release of cortisol or stress release kind of our stress response hormone. So when cortisol levels peak, so if you're in quite a stressful situation, your cortisol levels peak. This feeds back to the hypothalamus and your hippocampus in your brain. It shuts down their response, and like, this negative feedback loop. So they've shown in a lot of studies that if this loop or this access is impaired in any way, it can lead to severe chronic conditions, particularly with folks that we're talking about today. They've demonstrated that in more severe forms of depression, and also in anxiety or panic disorders, or obsessive compulsive disorders. There's lots of other pathways, so the vagus nerve is quite involved, they've done a lot of research into that as well. So this is the main nerve of the parasympathetic arm of the autonomic nervous system. So evidence for the involvement of the vagus nerve and mood disorders has been reported kind of 15-20 years ago in kind of more anecdotal observed in studies where they have patients who've undergone treatment for conditions of the stomach. So if they were treated for peptic

ulcers, they would sever the vagus nerve and that seemed to provide benefits there, but then they find with those patients, they demonstrated or reported back that they noticed they had depressive symptoms for the first time or increased depressive symptoms following the procedure. And there's been a lot of animal studies here as well, where they've severed the vagus nerve, and treated it with different types of probiotics, and lactobacillus rhamnosus being one of them, and they fail to show any anxiety related improvements. But they indicated that different bacterial strains independently can encourage communication between the gut and the brain. So when the vagus nerve is severed, they no longer communicated. There's lots of different reports for different bacterial strains. You can find a lot of them, but mainly Lactobacillus and Bifidobacterium.

And then there's again, another important mechanism for the vagus nerve is its involvement with microglia. So these microglia cells are quite important in terms of immune response. So the pro-inflammatory cytokines are producing this kind of neural signal that connects the peripheral nervous system to the brain. So the results of kind of activating these microglial cells, they're specialized macrophages, they essentially just clean up the brain. So any kind of damage, if there's injury, so injury through disease or pathology, so say like a stressor or an Alzheimer's or Parkinson's type disease, they're quite important in terms of maintaining that or, if there's infections in the brain, they play a really important role there, in managing kind of neuronal debt, degeneration of new neurons, and then creating new synaptic interactions. Stress has one really big impact on this neurogenesis, in terms of involvement of microglial cells, and they've done a lot of studies in germ free mice or mice who are kind of born in these sterile conditions, where they have defective microglial cells, and that they can modify those through the gut microbiota. So if they give

them dietary fiber, and they therefore generate short chain fatty acids, they see an improvement in this functioning of the microglial cells. Short chain fatty acids are another mechanism, so we know that short chain fatty acids, particularly, acetate, propionate and butyrate, they're the main metabolites produced in the colon, and they're also known to regulate physiological processes. Again, you'd mentioned the research that was conducted in Cork, so one of the earliest studies for the short chain fatty acids was published at the same group Timothy Dinan in Cork, and they've shown that the administration or short chain fatty acids administration is a potential treatment for different range of psychiatric disorders.

Another really interesting mechanism, tryptophan production, so essentially, tryptophan, the precursor serotonin, so an important hormone for modulating moods is increased in animals who are treated with bifido infantis, so another probiotic strain. And the brain kind of has limited capacity to store tryptophan, so it needs a constant dietary supply. So tryptophan comes in two main sources, the diet, I think one of the – one of the greater contributors would be turkey, and then certain bacteria can also synthesize or create their own tryptophan. So bifidobacteria can create their own tryptophan, so making it more available. So that's quite unique, that's another really important mechanism. And then kind of, finally, there is a little bit of evidence for neurotransmitters so the gut microbiota can also make brain active molecules such as serotonin or catecholamines, and there doesn't seem to be like a massive amount of strong evidence for this yet, but there is some evidence for probiotics and neurotransmitters and the secretion of neurotransmitters as well, but more in animal studies and linking that with different mood disorders, so again, depression, obsessive compulsive disorders and autism.

DANNY LENNON: Yeah, I think we'll probably be able to dive in to some of those interventions in some detail at some later point. I think maybe it might be worth taking a step back and a broader overview before we talk about to this interaction of either diet on the microbiome, and then therefore on mood and mood related disorders, or even probiotic supplementation, then having this effect through the microbiota on to some of these end outcomes we're going to discuss. I think even thinking about how diet plays a role on mood or mental health or any of these outcomes that we're looking at, generally, is actually quite tricky. This whole general field of, I suppose, nutritional psychiatry, we could call it, is quite interesting. So it might be worth actually explaining some of that difficulty or the challenges within that field. So maybe, Alan, for you, at least, when you venture into any of these looks into nutritional psychiatry and you're reading in this area, whether it's related to this topic or just more broadly that area, maybe what are some of the things that kind of highlight some of the complexity here, or the challenges that might be useful for people to know?

ALAN FLANAGAN: Yeah, I think there's possibly a few layers of complexity that make this area even more challenging than, let's say, for example, if we're looking at cardiovascular disease maybe or diabetes. One is that the, broadly speaking, most of our evidence currently, is in relation to overall dietary patterns. And so, what we tend to see is, you could, for example, have a relationship between a typical western dietary pattern, so low in fiber, low in vegetables, fruit, polyphenols, micronutrients generally, kind of, high in fat, high in refined grain and carbohydrate, all those characteristics of a Western dietary pattern, and we'll get these associations with depression, for example, as one kind of outcome. And you're kind of left shrugging the shoulders and saying, well, okay, but there could, again, also be a lot more going on that isn't solely related to diet. And a lot of research in this area, unfortunately, is not as

refined in trying to adjust for and account for a lot of these additional variables as you might otherwise like.

Conversely, on the benefit side, we have these associations with, for example, say, a Mediterranean diet pattern improves mood. And like, okay, this is, again, it's good for when we have diet pattern research, but it's not necessarily for how complex then, the kind of relationship between the microbiome and the gut brain axis is. People are trying to move from very general observations to very specific observations by taking some kind of large deductive steps. So there are some gaps, shall we say, between some of the broad associations that we have, and some of the kind of deeper understanding of how some dietary interventions might interact with the mechanisms that Niamh was describing to actually improve mood. Another challenge is in relation to what actual aspect of food related compounds are having a benefit on the brain or central nervous system and why. So a really good example of that, but I think we've discussed before on previous episodes is with flavonoid compounds, and their interaction with neural outcomes, generally speaking, improving, for example, neuroinflammation, evidence for influencing pathways involved in learning and memory, and these general benefits to cognition. However, if you only studied the parent compounds, you would see that they have quite a short half-life. If you study the metabolites of these parent compounds, you'd see the metabolites are in circulation for much longer. And then the second part of this that's important is the blood brain barrier, and the blood brain barrier is this kind of layer of, kind of, it’s semipermeable layer of cells, essentially, a border that prevents certain substrates in our blood from crossing into the central nervous system. And it's very good at letting things in or keeping things out. And a lot of, if you looked at, for example, sticking with this parent compounds, you'd see that actually they don't cross the blood brain

barrier in much quantities, if we were, say, to talk about anthocyanins or other compounds. And so, that leaves people with the mechanistic question of how are they having effects in the central nervous system or on cognition. And the answer to that is, they appear not to necessarily have to swarm over the blood brain barrier to have an effect. A lot of these compounds act through signaling pathways, so they bind to these kinds of protein signaling pathways, and they influence a particular pathway. So, for example, brain derived neurotrophic factor.

So it's possible that we have compounds in the diet that don't have to necessarily, that are metabolized in the gut by the bacteria that we have. Those metabolites are absorbed in greater quantities than the parent compounds that we've consumed. But the gut microbiota plays a vital role in the production of those metabolites that are then absorbed through, for example, enterohepatic circulation. They're prolonged in circulation, but they're not necessarily having to cross the blood brain barrier in great amounts to have an effect because of the various pathways and mechanisms that Niamh was describing, and some of these other mechanisms that they're actually acting through signaling pathways. And so, that could be important, for example, when we think about a compound like tryptophan, like, we know that tryptophan doesn't cross the blood brain barrier in significant amounts, but we do know that there is some activity of certain enzymes in the microbiota that can result in the influence of certain pathways, that may lead to more serotonin production, as opposed to this other pathway known as the kynurenine pathway which kind of steals tryptophan away from that production, and actually is associated with kind of proinflammatory and other outcomes.

I think to kind of distill this, there's probably three layers, one is that a lot of our evidence is very general, as it relates to kind of food and

mood, is very general and relates to dietary patterns overall. Secondly, is that when we do go beyond dietary patterns, and start to look at the level of particular nutrients or compounds in the diet, that might have a benefit, then there's a lot that we have to kind of evolve in terms of our understanding of the pathways through which they act, and they may not necessarily reflect some of the kind of traditional mechanisms that we would assume that nutrients kind of act in, and a lot of that may have to do with their interaction and metabolism by the microbiota itself. And then, of course, we have the effect of diet itself on the composition of bacteria in the gut, and those bacteria themselves may influence through these various mechanisms that Niamh outlined at the start, some of these processes in terms of the gut brain access in the enteric nervous system. And so, from a methodological standpoint, there are issues with defining the exposure, there are issues with the selection of participants, in particular, what kind of condition they may have in the first place, and how serious it is on whether it's kind of mild depression or like moderate depression or psychosis. There's issues then in terms of factoring in some wider covariates that you'd really want to account for, like, concomitant, for example, cognitive behavioral therapy, adjuvant to the intervention, it's often not accounted for, or selective serotonin reuptake inhibitor use or other psychotherapeutic drugs. And there are just kind of within with the field this new as well issues in relation to the size of the studies, the duration of the studies. It’s a novel emerging area, so there's some of those kinds of general limitations that we would see with novel emerging areas of nutrition science, where it's preliminary evidence in humans, in small studies that are weakly powered, and some of them have odd outcomes, and there's not a lot uniform there. So it’s a really interesting area with a lot of promise, but one, which, in terms of human evidence, right now, there are possibly more open questions than closed answers.

DANNY LENNON: Yeah, I think just one in addition to that, that I've discussed recently, particularly, when it relates to something say like depression, which is obviously incredibly complex, multifactorial, a lot of maybe trials are looking at people that have had depression for a long period of time. And then we're using an intervention that maybe we're expecting a bit too much from, depending on the type of condition we're looking at. But also, like you say, because of whether it's the duration of the trial, or even the duration of follow-up, now you can have a really difficult issue of, if you've had someone with a chronic condition like a depression, and you have a very short follow-up time, after the study is over. Can you really draw conclusions over how much that has helped, and how transient maybe that effect was, etc., and that's not a point about specifically nutrition or what we're talking about today, it's really kind of any of these interventions that are being looked at for something as complex as that. But yeah, there's a lot going on there. I don't know if any of that triggered off some additional thoughts you wanted to throw in Niamh, before we move into the studies?

NIAMH ASPELL: No, I just agree with it all, and it's kind of everything that I was really surprised, looking at the clinical studies that have been, a lot of them that have been conducted so far. And what they have seen to omit in their study design is quite – it was quite surprising. So previously, I had worked on interventions where I was designing studies, looking at different probiotics and their outcomes and different things such as mood. And these are really, really well designed studies, which the kind of components of that would add a minimum, baseline taking a stool sample to check what their microbiota looks like before the intervention, take it at the end. Most of these studies didn't do that, they also didn't do any dietary assessment. So we know how easy your gut microbiome can be influenced by different drugs, different diet, dietary patterns,

sleep, travel, exercise, none of them mentioned any of this in any of their assessments. It was just very kind of crude markers of the, you know, they are validated tests in terms of measuring anxiety and depression, but they are very short term tests, and they don't consider any other quality of life measures. A lot of them have these kind of dual design where they're looking at, like, irritable bowel symptoms and mood, and they're showing that the probiotic has a beneficial effect on their quality of life, but it's their IBS quality of life. So, of course, if you improve their IBS symptoms, they will more than likely feel better, but they're going to report this as overall kind of mood and depression. But it's more a secondary outcome of improved kind of physical health. So there's lots, yeah, there's lots of things that I was really surprised about, and my PhD focused on looking at nutrients and cognitive and psychosocial and psychological well-being. And one big part of that was to try and understand other serum markers, like B vitamins, which have some demonstrated evidence in adult populations, and vitamin D, to kind of relate some of the other nutrient interactions that could be contributing here, and none of them did that either, so I was quite surprised that on the quality of them. And again, yeah, just the sample size, they're more like pilot studies, not many of them are powered to really show anything. And again, probiotic and inpatients are inpatients with severe episodes of depression. If they're not responsive to medications, how likely are they going to be responsive to a probiotic supplement for a short duration, and they don't seem to have followed them up after the intervention to see, well, if there is a positive effect, does it end the day after you stop taking your probiotic, or, does it continue, is this like a continuous treatment.

ALAN FLANAGAN: And that's the thing, which, if we're just thinking about wider probiotic research, not necessarily, and with a range of outcomes, whether it's kind of IBD or IBS, one of the

things, and you mentioned that the gut – we know that the gut microbiota shifts very rapidly in response to diet, I mean, you can see that in interventions, just looking at diet on the gut, in as little as three days, depending on how extreme the shift, particularly in either carbohydrate, fiber, and dietary fat is, depending on how extreme that that shifted diet is, you can see quite profound changes in as short as three days. But what you tend to see is, as soon as the intervention is ended, you get a return to the baseline microbiota composition, just as quick. And so, one of the wider ideas in the general diet microbiota research is that once you are an adult, essentially, you have a fairly stable microbiota over the course of your life, which can have short term responsiveness, but overall long term, relatively kind of robust to major changes in the composition, save some sort of... And so, there is that real question that needs to be asked in terms of, we can't just assume, based on that other research, that the intervention that may have found whatever benefit over a course of a number of weeks, would be a sustained improvement in that outcome, given what we know so far, in terms of how rapidly it could just shift back to whatever their baseline was. But, as you say, they don't really do any baseline or follow-up measures of the microbiota to allow us to actually see what kind of changes have occurred.

DANNY LENNON: So maybe we can mention some of the specific studies that might give people a good example of what we've discussed, because obviously, there's a lot of mechanistic data in this area, a lot of that early work, and even continued now that has been really exciting for good reason has been in various rodent models, and we can even maybe mention some of that. But if we put our focus towards some of those human trials, and we've kind of talked a bit generally about those, is there any, in particular, that might be a good example to bring up that we should get into particularly or that jumped out

at either of you as we were going through some of the background for this topic?

NIAMH ASPELL: Yeah, I can start with – there was one published back in 2018, I think we've kind of touched on this a small bit. So this is a probiotic treatment as an adjuvant therapy to try and prevent rehospitalization in patients who've got acute mania. So this was a randomized controlled trial conducted in the States, and it was published back in 2018. All of the papers that I looked at, or all of the studies I looked at, this one was kind of one of the more promising kind of human intervention studies. So it focused on exploring the potential, it focused more on the immune kind of modulating effects between the gut microbiome and the brain. And they supplemented participants, really small number of patients, but when it's a patient population, it's a little bit more challenging, but they supplemented with lactobacilli and bifidobacteria probiotic different strains in adults who had recently being discharged from hospital with mania. So these patients had recently had an event essentially, and they were discharged, and they were randomized onto this trial. So they were either given that combination probiotic or a placebo, and they wanted to determine if they administered these particular probiotics would prevent psychiatric rehospitalization, so presenting again with similar kind of symptoms. It was relatively okay duration of study, so it was a six-month intervention, so we know that'd be kind of long enough to create change in terms of the microbiome.

In terms of follow-up, I'm not actually sure what the average follow-up would be in terms of rehospitalization for mania, but I think six months seems like a reasonable kind of follow-up. But they recruited 66 patients, so it was quite small, but they managed to retain most of the patients for the period of the intervention, which is really good in a patient population; and they gave them either the placebo

probiotic. The only thing with this study is that the patients are all on different pharmacological treatments as well, so not standardized pharmacological treatments, they were on whatever they had initially been prescribed for their condition. And we know different pharmaceutical agents, particularly, well, kind of, particularly antibiotics, but a lot of agents as well that are prescribed for depression. We know lithium has a positive impact on gut microbiota, but it's been reported that up to 80% of all prescribed drugs impact the gut microbiome in some way, and the impact can be either negative or positive. So this study didn't control for that, they had patients who were receiving different forms of medication. So that is, obviously, quite a flaw in that regard, but the authors do acknowledge that. They also looked at trying to analyze the risk of hospitalization kind of over time, and they looked at systemic inflammation measures, using different blood markers. So even though there was a small sample size, the results were quite pronounced.

So during the six month intervention period, a total of 24 rehospitalizations occurred in 33 of the individuals who were on placebo. So that's a very large proportion of that part, that cohort in the study, and that was compared to eight rehospitalizations in the 33 patients who received the probiotic. So the probiotic treatment also resulted in fewer days of rehospitalizations for those patients who were rehospitalized. So a comparison of an average of 8.3 days compared to 2.8 days for the probiotic group, so they obviously were in hospital for a shorter period of time. There's very little reported in the publication around additional events in the kind of period before hospitalization and rehospitalization, so we don't know much around the circumstance of rehospitalization. But the study was quite well designed. There's those limitations around the pharmacological aspects, and also they didn't conduct any analysis on the intestinal microbiome before and after the use of the

probiotic, which I think is quite a big limitation. And some of the measures they use are quite crude, I think, looking at the inflammatory markers are quite interesting, but these are circulating inflammatory markers as well. I think measurement of inflammation in the central nervous system is a little bit more invasive. So if it was like cerebral spinal fluids, that could be a possible technique, but obviously, you have to think of the ethical practice of conducting an invasive technique like that when somebody's been rehospitalized, particularly for the condition where we're focused on. The authors reported as well that they didn't find a difference in the average level of psychiatric symptoms measured when they assessed them at monthly visits. So at monthly visits, both groups were reporting kind of similar – there's no massive change in their symptoms, so they didn't really understand totally what caused that, what event or what led to that kind of event of the rehospitalization as well. So still understanding the complexity of the condition as well isn't really well kind of pulled apart in the study, and kind of go back to all those indicators that you've mentioned as well, Alan, around things that really contextualize the event and the circumstance as opposed, you know, even though the results are quite strong for the probiotic, we know very little else around anything else in terms of the patient's circumstance.

ALAN FLANAGAN: Yeah, and I agree, I think this is one of the bachelor designed interventions, human interventions overall, compared to a lot of the others, but still then kind of leaves you slightly disappointed that some of these extra measures weren't taken. I mean, it's a 63% lower risk of rehospitalization in the probiotic group, which has an enormous effect size, and again, small study, 33 participants in each group, the intervention probiotic and the placebo, but that's still an enormous effect size for nutrition, kind of, related exposure, not a very precise estimate, but still quite large. And one of the things that struck me, and again, not really

knowing psychiatry well enough, but one of the things that I noticed that the groups weren't particularly well balanced for, was their actual DSM-5 diagnosis where the placebo group, 73% had manic bipolar, whereas 52% of the intervention group did. Now, is that particularly relevant? Would that have led to or contributed to the lower risk of rehospitalization in the probiotic intervention group? I don't have enough of that domain specific knowledge to know. But just from a methodological perspective, it was something that I kind of was like, oh, this would be something I would be wanting to ask someone in psychiatry, like, hey, would this be something that could play out. But yeah, I think overall, it is still one of the better studies in this area, but it has, as far as human studies go, but it has these couple of limitations that makes you then left with more questions as to why there was such a pronounced reduction in risk in the probiotic group over that 24-week period.

NIAMH ASPELL: Yeah, I wouldn't have the knowledge to give any input in terms of the psychiatric condition and the involvement there. I think one important kind of thing as well to acknowledge is that they don't, in terms of the patient's condition, they don't discuss other support mechanisms beyond the pharmacological treatment. So we do understand the circumstance and the ability to recover and adapt for that patient once they leave hospital, so what support systems are in place, whether it's social supports, there's a lot of research coming out of the University of Oxford, where they have been able to identify certain personality traits and their impact. so things like people with larger social networks or greater support networks have more diverse microbiome we know. We know in certain mental health conditions or mood disorders as well. Your social network is extremely important. Sociability is important, but then other socioeconomic factors. So in terms of, okay, you've left hospital, are you able to return

to work? If you're not able to return to work, are you being supported? So a lot of those kind of factors aren't considered at all. And I think that also would be like beyond the diagnostic, the clinical diagnostic criteria and the imbalances there, they need to kind of also explore what other external supports that the patient have.

DANNY LENNON: That study there we have something where we have a kind of clear diagnosis of a psychiatric illness or some of the others didn't have maybe more general, kind of, I suppose, descriptors of mood state for people, and there's one I know, Alan, now that you're a Yakult influencer, I don't know are you contractually obliged to bring up the Benton study, is that – do you have to bring that up?

ALAN FLANAGAN: I have a conflict of interest. I had one when I got back in the door, and I've really had a second one now, and Dr. Shirota has boosted my mood. So yeah, I mean, that's something that I think is interesting, yeah.

DANNY LENNON: Yeah, you said this was the greatest study you've ever read...

ALAN FLANAGAN: Ever read, yeah. There are no conflicts of interest. It's not industry funded. And basically, everyone should go out and buy Yakult immediately.

DANNY LENNON: For COVID prevention, I hear, you were talking to me earlier.

ALAN FLANAGAN: Yeah. I've been speaking to a few doctors in the States, and we're really pushing to replace Ivermectin with Yakult. So for listeners who don't think this is beyond a little bit weird, when I was a kid growing up in Hong Kong, Yakult was like all the rage, and I didn't even know that the term probiotic existed when I was seven. So to read the study where the exposure of interest was actually Yakult was rather amusing.

DANNY LENNON: Yeah, so for maybe for people who don't know what that is, that's a branded yogurt drink with probiotic.

ALAN FLANAGAN: Yeah, it's like a little skimmed milk based drink...

NIAMH ASPELL: Sugar syrup.

ALAN FLANAGAN: Sugar syrup drink with a particular...

DANNY LENNON: It's like a shot of heaven.

ALAN FLANAGAN: Yeah, just get out there and get it. And it's got a very specific strain of Lactobacillus Casei that, I believe, some Japanese researcher found, and is supposed to be good for the gut, but who knows in humans, but we do have this intervention. It's one of the older ones, it's a 2006 study from Benton, Williams and Brown that was in the European Journal of Clinical Nutrition, and it's called Impact of consuming a milk drink containing a probiotic on mood and cognition. And I guess, perhaps the reason I was surprised was some interventions have used specific kind of formulations of essentially like a fermented drink with added probiotics, whereas this was using a commercially available probiotic drink that they, to be fair, they attempted to then match placebo for kind of taste and tested the participants using kind of visual analogue scales on a range of kinds of taste parameters, to see if they noticed a difference with the placebo, and there didn't seem to be any difference at all. But this was a study in the general population, around 132 participants recruited overall, 124 completed the trial. And what they did at baseline was they did a range of kinds of them questionnaires for behavior, some assessments of things like memory, and they also then randomized patients to consume this Yakult or the placebo on a daily basis. So they would have one a day, and they consumed that for 10 days, and then they self-reported mood using a visual analogue scale.

So for listeners, a visual analogue scale tends to be if you just imagine a horizontal 10-centimeter line on a page, and it has a verbal anchor attached to either end of that line, and so, that might be, for example, if it was hunger, for example, it might be as hungry as I've ever been, or cannot eat another thing or these kinds of verbal anchors. And then people literally cross with a pen or pencil the line, and then you would take a ruler and measure where that is. And if it was 7.2 on your 10-centimeter scale, then that would be your actual numeric value for whatever it is you're measuring. And so, you can use visual analogue scales for hunger and appetite, and you can use them for mood scores, and you can use them for a range of things that have been validated. So they use that to assess mood, and this also related to – they were looking at constipation as well, and so, seeing whether there was an improvement in bowel movements per day in participants. And they drank the drink for 10 days or in the placebo, then they repeated the lab based cognitive and mood tests, they had taken a baseline and they consumed the probiotic then for another 10 days. And then, they repeated the tests then again at day 20.

So it was a 20-day intervention overall, and they looked at these kind of, like I said, eating behavior, some memory outcomes, and then a series of kind of mood related outcomes. And what they found overall was, well, kind of, very little. What they found – and they did a subgroup analysis, but like, again, it's like, still a small enough study overall – what they found was there was overall very little difference, like, no effect of the actual probiotic supplements. But they had a population that seemed in terms of their baseline questionnaires to have, like, a fairly upbeat outlook on life, and fairly good mood overall. So they thought, okay, maybe that's potentially a masking factor that would hide any effect of the intervention. So they looked specifically then at patients who, they divided patients into kind of three categories of mood at baseline. And what they did find was

that the people in the bottom category did have some effect in terms of increased self-reported mood, better mood, i.e., specifically happiness after taking the probiotic. But really, there were no other events or outcomes really of note in the study. It didn't have an effect on bowel movements, and there was actually some suggestion of potentially a decreased performance on some of the memory tests, but I wouldn't – cognitive testing and research is, like, particularly with nutrition exposures, as Niamh would know very well, can be fraught with some challenges as far as the kind of integrity of the tests conducted in studies, and I wouldn't exactly be suggesting that the little sugar drink was making people less verbally fluent in this study.

So really, it's quite a giant null overall with the potential positive effect in people who had low mood at baseline, but again, the problem with these studies is the range of factors in any given individual's life that could constitute increased happiness. In the broadest sense of the term, over a 20-day period, and with the lack of really any kind of more sophisticated control and assessment, it's difficult to come away saying, oh yeah, it was that little sugar shot, skim milk sugar shot with a bacterial strain, and that did the happiness boosting. So interesting attempt and again, I think the whole path potentially for this study is, it is 2006, it's possibly one of the first studies that really sought to conduct a human intervention with a probiotic as an exposure of interest in mood related outcomes. But it doesn't really give us any sort of overwhelming thumbs up for L-casei Shirota.

DANNY LENNON: Yeah, this is one of the studies that is mentioned in the statement, and one of the things that might be just interesting, more from a kind of discussion around research methodology, and for people learning to critically appraise studies that you mentioned in there, and even that statement was with this study, you brought up issues around repeated

learning and ceiling effects and those cognitive outcomes. Can you maybe just explain those concepts in the context of this study, but then, I think also, more broadly, might help people if they haven't came across that type of idea?

NIAMH ASPELL: Yeah, I think this is a really good example of it, I think a three-week intervention for cognitive assessment in a healthy population who are all reporting quite good mood at the start. A lot of the memory tests they use, I think, a well shared learning score, typically, for a population, a young population or population that are cognitively well, you would use a more complex battery of cognitive tests. So you wouldn't just pick a single domain and kind of category, so things like memory. You'd look at things a bit broader, so maybe like executive functions. You might do some more kind of imaging tests or functional MRI type tests, so you can detect smaller changes. You wouldn't really detect or particularly, not clinically meaningful changes in any of these kinds of parameters, using the scales that they've used. In terms of practice effects, there's a couple of things there, which I just remember about a study that, as part of my PhD, I did cognitive assessments with older people, all over the age of 65, who were cognitively healthy. And the intervention period was six months, and we chose six months as the minimum period, in which we could repeat the cognitive tests so that they wouldn't remember the answer or learn the answers in the same test, and these are really proactive participants. In about six months, they'd come in to me, and they'd be like, I remember all the words before I'd say them. And I was like, no, this is not like don't forget about it try and take that out of your mind after the first test. There is a way to overcome it, where, on the first visit, on the first baseline visit, for a cognitive study, you do repeated tests, so you'd get them to do the counter test three times, so you get the learning effect out of the way, and then you do their first test, and then you repeat it again.

So what I have found for healthy populations before, they scored really well in these tests, and there's no room for improvement, so that's when you have your ceiling effect. So you're kind of like, they do their first visit, they get a 100% in kind of memory score, and I'm like, the supplement, whatever it is, cannot bump you any further, you're going to come back in six months' time, you're going to remember it. And so they're very, very kind of crude measures. They probably are a little bit more beneficial to be used in populations where there's mild cognitive impairment, where you'd expect some more changes. But in a group like this, I think a lot of these clinical studies don't have clinical populations, and they also have people who are too well, kind of, to begin with, and I don't think that's where we'll kind of see a supportive change. And if we're looking at more preventative nutrition strategies for cognitive health, start in midlife, take the supplements, change your diet, whatever it might be, that's supposed to benefit, you do that for 20 years. Let's see in 20 years if your overall nutrition and lifestyle has improved, whereas I think healthy populations for three weeks, I don't know what you'd expect to really see. Also, they didn't collect any other circumstantial information around kind of alertness, sleep, circumstances around the time period of reporting. So in the space of three weeks, you could feel great on a Monday, come back three weeks later, you've just had a crap weekend, and you're like, now I'm picking the sad face on the analogue scale. So they didn't capture that as well. So there's lots of challenges with cognitive tests. You need to include a psychosocial assessment on top of the cognitive assessment, and do more kind of computed measures like attention tasks where you're repeated to do a task and it's computed and you have standardized scores, so you can detect really minimal small changes. Whereas some of these measures on their own just, they don't really stand up to what they're testing.

DANNY LENNON: Yeah, and that's one of the ideas that we're mentioning at the outset of selecting the right outcomes is important here, because sometimes I think there's, for obvious reasons generally with nutrition, we try and look at, like, actual changes in an end outcome that we care about. But sometimes that falls down, if you're trying to detect something where, again, maybe expecting too much from a probiotic supplementation over a small period of time, and if we're – maybe there are these small changes in certain measures, but we're not looking at them, we end up looking at these two grandiose outcomes. And as you outlined, then you end up missing some sort of effect, where in likelihood to actually change something as complex as, let's say, depression or chronic anxiety, it probably seems like yet, just a small nudge in the right direction is going to be clinically meaningful, because then you'd stack on top of other range of interventions that would be used in clinical practice, presumably, to have some effect. So yeah, it's the selection of outcomes is quite an interesting one.

ALAN FLANAGAN: You can end up with all of these, like, essentially proxy or secondary outcomes that get hammed up, and make it look like there was some sort of, kind of, major effect of an intervention, but really, it's just a kind of a scattering of proxy and secondary outcomes. The frustrating thing for some of these interventions is, yeah, and, I guess, maybe it goes both ways. I mean, in nutrition, we tend to give out when we see poorly conducted nutrition studies, and then you look at the author affiliations and realize none of them are even in a nutrition department. You're kind of like, well, maybe there's a touch of that going on, on the other side, where you've got these kinds of nutrition people doing these kinds of, essentially, very psychiatric interventions, and not accounting for a bunch of stuff that someone with domain specific knowledge in that field might be like, what are you doing. But I think, as a minimum, going forward, a lot of these interventions are really going to have to

standardize as part of, and see the nutrition exposure, whether it's probiotic, for example, or even a food based intervention targeting mood is except that the role of the nutrient or probiotic is adjuvant to everything else going on, and have all of those factors controlled for between your exposure and your control group. So they're getting the same CBT, or whatever the kind of behavioral therapy is, with the same frequency. They're relatively on the same drugs, they're matched for those variables as much as possible, and then it's like now one of you is on a probiotic, and one of you isn't, and I think unless we have that rigor of the additional control, in an area where we know so many of these other variables can often be the most important.

I remember having a chat with someone who was in psychology, but very interested in nutrition, kind of, talking about the SMILES trial and some of the other studies. And one of the points he made was that if you look at any sort of psychiatric or psychological intervention, the biggest predictor of benefits is not necessarily the specific modality, it's the number, it's the intensity of engagement with the practitioner, right? It's number of practitioner contacts is the biggest predictor. So just going to therapy versus not going to therapy, and then going to therapy more versus going to therapy kind of less, like, that's generally whether they're doing CBT or another modality, that's generally associated with the outcome. So if factors like that aren't in the protocol as part of the overall control, then I don't know that we can come to much conclusions by way of the specific probiotic strain or the specific food based intervention until that level of rigor for these other variables from a psychiatric and clinical psychology prospective are controlled for between our groups.

DANNY LENNON: So if we take that almost as the kind of concluding line after going through this, are we kind of left with a situation of, if someone to

ask for anything related to either improvements in mood for people generally or actually something like anxiety, depression or any other psychiatric illness, does probiotic supplementation have an effect in humans – are we kind of left to conclude, well, right now, we don't know? Or how would you guys tend to sum up the evidence may be more nuanced than that?

NIAMH ASPELL: When I worked in Atlantia Food Clinical trials back in Cork, we did a lot of work with the APC and Cork as well, and the medical director in Atlantia was Ted Dinan. So I worked with him quite a bit in designing the studies and he obviously is very interested in the potential of psychobiotics and their use in depression or milder forms of depression. And he would be very, very honest to say that we still don't understand which strains, we don't know the quantity of those strains, and it's very unlikely that they will have a massive impact on severe forms of mood, depression. It's more likely that it will support patients who maybe don't want to take or don't necessarily need anti-depressive medication, and it might be used in their kind of toolkit in terms of how they manage their mood or their mental health. But he'd also say that we wouldn't look at probiotics in isolation, that it has to be in conjunction with diet, aerobic exercise, and other kind of health and lifestyle behavior. So I think for me, that would be my feeling as well, particularly from somebody who, so knows the area so well, but also his research agenda is obviously driven by probiotics, and he's termed or coined psychobiotics, so he's pushing and driving that. But he would say himself, it's not going to change your world, you need to do all of these things, there's potential there, we still don't understand it. And the interventions I know from been involved in the last couple of years are being designed in a much, much better way than the ones that we talked today, so I think in the next couple of years, there is potential that we will understand this a little bit better. But for now, if somebody came to me

and said, oh, should I take a probiotic, I'd be like, do you want to spend 30 quid a week on a probiotic, or do you maybe have a look at what you're doing at the weekend, and maybe change that a bit, and maybe, like, go for a jog or do these other things that we know, can affect or improve or support kind of good mental health. I think it puts a lot back on the patient as well to say, kind of like, oh, just take a probiotic, you know, it's back to that kind of, oh cheer up narrative kind of thing. So it's universal therapies and consideration of the environment.

DANNY LENNON: Yeah. And, I mean, it still leaves things of like, like you said, if it is around mental health, there are interventions that we know are beneficial, whether that's like through diet and exercise I'm talking about now, that we know can be beneficial there. But we also know clearly they impact the gut microbiota, and then if you're left in a situation of kind of, well, we still need things to figure out, it's probably preferable to do those things that would support what we would think of as generally healthy microbiota versus not. And so, there are certain types of dietary patterns, as Alan mentioned, that someone can follow to do that, it's probably good to engage in physical activity. There's these other things that we do know that people can engage in, as opposed to saying, oh, well, we don't know anything, let's forget about all this stuff, all this area is nonsense, whereas it's actually not, it's just like, there's still some things that are unanswered, I suppose.

ALAN FLANAGAN: Yeah. I think that's – there's something, when it comes to the mood and the psychiatric kind of side of things, there's something really hesitant in me about saying anything kind of about food per se, knowing that so many factors can affect someone's access to good nutrition, and particularly after some of those kinds of food based mood interventions came out, I thought my sense was that they were really overblown, and there was a lot of

overenthusiasm for what food was doing, and what people were saying food was doing versus what really was potentially, as part of an overall model of an individual's improvement. And so, there's a hesitancy that I have to say, yeah, like, eat more vegetables and your mood will improve. It doesn't sit well with me as a statement. So I think we need to be really careful of that, and I think, generally, people kind of are. I think it's more kind of out in the kind of lay public space that you get a lot of that kind of push on your food cures. I think to some, like, yeah, absolutely agree with everything that has been said there as kind of far as where we're at with the field. I do think that, yes, generally speaking, improvements in overall diet quality and certain aspects of what we already know do benefit certain strain specific populations that in the gut that we know. So, for example, increasing dietary fiber, and a kind of an array of fiber sources like prebiotics, non-starch polysaccharides, and these kinds of compounds, beta glucans in oats, that we have evidence already, increase beneficial populations of bacteria in the gut. And that's generally associated with a healthy microbiota, even though even that's quite an opaque concept right now in and of itself. And I think, perhaps just really, that's as far as we can go in terms of the specific microbiota, rather than kind of getting into, well, what strain benefits what, because as we've learned from other conditions as well, the benefit of probiotics often appears to be very strain specific, but also specific to particular conditions. So I think we're still a way off, as Niamh said, like, better interventions being designed, and it'll be really interesting to see those emerge. But when it comes to kind of like the psychiatric, the neuropsychiatric side of things now, I think nutrition can be an important aspect to improving that overall picture, but in a pecking order of priorities, in terms of, say, actual engagement with therapy, or potential pharmacological interventions, or sleep patterns, all of these other kinds of variables. I'm not sure that I'd put nutrition at

this point, unless diet was really poor quality overall. I'm not sure that I'd put nutrition with those other interventions at this point.

DANNY LENNON: Yeah. No, I very much agree. It's one of my many things I hate about the fitness industry is seeing people that you probably know, certainly have no real first contact with depression, either themselves or someone they know or really no grasp of it, certainly haven't engaged in any therapy, but then are talking about the things like, oh, if you go in a certain type of diet, or just start training, then that's kind of like a prevention or a treatment for depression. And it's kind of this nonsensical thing of like, I don't think that's true at all.

ALAN FLANAGAN: Yeah.

DANNY LENNON: There's so many people who could have an ideal diet if there was such a thing, and yet, we'll suffer from a variety of different psychiatric illnesses or mood related disorders. So I think, yeah, any simplistic narrative is problematic, and I probably wouldn't agree with Shawn Baker that a carnivore diet cures depression.

ALAN FLANAGAN: Yeah.

DANNY LENNON: Yeah, it's funny. I was talking recently, just messaging with Kevin Klatt, if people don't know him, he's a registered dietician, but also a fantastic nutrition researcher. And we're just talking about some of these variety of nutrition questions that can come up where, when you try and get to like a really firm, evidence based answer, you kind of are left saying that, well, we kind of don't really know, and he said to me, and I thought it was brilliant, he's like, it should really have like a nutrition podcast and call it, We Probably Don't Know, and it might be a perfect answer for so many questions.

ALAN FLANAGAN: Yeah.

NIAMH ASPELL: Yeah. I think Ben Goldacre has a great book on that, where he's like, I think you'll find it's a little bit more complicated as the title of the book, yeah.

Alan Flanagan Yeah.

ALAN FLANAGAN: Yeah, when you read these, the titles are amazing, they're great. And you read them a little bit more, and you're like, you've considered nothing. But I think, yeah, once the evidence gets a little bit stronger, I think there is potential for probiotics and prebiotics. But like you said, it's a building block on top of so many other factors, and it's not – it's definitely never going to be a silver bullet, I don't think.

DANNY LENNON: Yes.

ALAN FLANAGAN: Yeah.

DANNY LENNON: Awesome. Has there been anything that we didn't get to that you think is particularly important, or any loops that we've left open, or is that kind of a good note to finish this on?

NIAMH ASPELL: Yeah...

ALAN FLANAGAN: Yeah,

NIAMH ASPELL: Okay for me, yeah.

ALAN FLANAGAN: Yeah.

DANNY LENNON: Awesome. Okay. We will leave it there.