DANNY LENNON: And so, maybe as a way to introduce this topic, so if we're thinking about, first of all, what are polyphenols, and then how does that fit into what are phytonutrients because we've, of course, mentioned them on the podcast before as well, simply we know that beyond the vitamins and minerals we're consuming, that there are compounds contained within plant foods that have a potential positive impact on health; and a number of these compounds, we can group together under this name of phytonutrients or sometimes phytochemicals. Now, you may hear some people reference them as not being essential nutrients, and indeed, that is technically correct, they're not essential in the way that certain vitamins and minerals are, but that is not essential for survival reasons, as opposed to what is good for health; and that's a distinguishing thing that we'll probably circle back to throughout this discussion. There's tens of thousands of these phytonutrients at this point, and one of the interesting things again, that we might reference later on is that not only is there are tens of thousands of these compounds, but each one of those then has a variety of metabolites that are going to appear then in circulation after they came into the body. And so, because there's a number of metabolites per

compound, we could have a huge number when we include both the parent compounds, but also these metabolites, so tens of thousands, if not hundreds of thousands of these to consider.

Now we're going to be talking about different classes of polyphenols throughout this discussion. So polyphenols being one type of phytonutrients, and within polyphenols, there's a number of different subclasses that are based on essentially the chemical structure of those. So we could have phenolic acid, we could have flavonoids, and they'd be the two main groups we're going to look at. But within that, we have these different subgroups that we're going to mention, so hopefully, it doesn't become too confusing, but just know that we're working down from polyphenols, different subtypes of those, for example, flavonoids; within that there's different sub types of flavonoids; and then within those, there's different individual types of compounds, which we'll hopefully try and explain as we go through. And in most cases here, when we're talking about polyphenols, they're going to be concentrated in plant foods, like we said. There's one really interesting paper out of France that we'll link to in the show notes, a 2010 paper, where they created a list of the top 100 foods in terms of polyphenol content. And they use something called the phenol-explorer database, which will also rear its head in a couple of the papers we may mention. This is a huge comprehensive database that anyone can go and access, it's phenol-explorer.eu, and there they've basically taken every food and quantified the polyphenol content, the types of polyphenols in all these different foods. So using this particular paper saw that these riches sources are things like various herbs and spices, cocoa products, darkly colored berries, some types of seeds like flaxseed, some types of nuts like hazelnuts, vegetables, and then things like olives and so on.

So, again, an umbrella term for a variety, a large amount of different compounds that can

then have maybe slightly different effects, but we're going to use this broad umbrella term of polyphenols; and then we'll probably specify different types of polyphenol throughout this course of this episode as well. So hopefully, that serves as a bit of an introduction. If there's anything there that we should add, do you want to add to that, Alan?

ALAN FLANAGAN: There's the risk with this topic of deciding to go down a kind of rabbit hole of the chemical structures, which differ I think, for actual present purposes, I think just clarifying what these compounds are in terms of bioactive, non-nutritive bioactive food components, and then, yeah, having that broad class of polyphenol within which phenolic acids and flavonoids and then within flavonoids, we've got various different subtypes. I think as a kind of mental model of a tree, a family tree, so to speak, that's probably the best level to keep it at, because it's sufficient to actually think of the exposure at this level.

DANNY LENNON: So I think before we discuss any of the cohorts that we're going to look at or any of the intervention studies, we thought it would be best to look at the mechanisms and the biological plausibility or, essentially, why would we hypothesize there is a benefit, and specifically today, if we're thinking about things like neurological health and cognition, why is it that we would be investigating the role of these bioactive compounds. So of course, this could become a huge area in and of itself, but maybe if we keep it to the most kind of salient points, what are the first things, I guess, people should know about mechanistically why we're thinking about dietary polyphenols?

ALAN FLANAGAN: Probably the best point of departure is their metabolism, because it's intrinsically linked to their actual mechanisms of action. It's also something that has been quite important in terms of marrying effects, both from long term prospective cohort studies and short term studies, with some biological plausibility. As

you mentioned, polyphenolic compounds are not nutritive, and so, this is quite important to their action, because they're essentially metabolized as drugs, they're treated by the body as xenobiotics, and they're metabolized through the liver in the same way that drugs would be. And so, this is important, they're also then, if they pass to the large intestine to the colon, they undergo extensive metabolism by the colonic bacteria, this is also really important to their ultimate mechanisms of action. So this is this crucial first step, because it produces, this metabolism produces this wide array of different polyphenolic metabolites that are then released back into the circulation or absorbed into the circulation through the hepatic portal vein, and they can continue to be recycled extensively, even up to 20 times. And the metabolites maybe up to a 100 fold greater in concentration in circulation than the parent compounds themselves, and they have significantly higher bioavailability. So you can get some uptake from the small intestine which is one pathway, and then they undergo metabolism in the liver through what we would know as first pass metabolism. And then they can also pass to the colon, like I said, and then undergo this rapid or this degradation by gut bacteria into these metabolites, which are then absorbed in quantities that are far greater than the parent compound itself. And then finally, like we mentioned, there's enterohepatic circulation, so the hepatic portal vein, the recycling of compounds between the liver and the digestive tract, and this is another root of polyphenol metabolite circulation.

So this is where it gets pretty complex. So if we have anthocyanins, for example, which are a class of flavonoids, you could have the parent compound. Now initially, research looking at just the parent compound showed that its half-life was about 90 minutes. So that made people go well, how could there be a chronic benefit to these compounds, how could we see a benefit in a cohort study going over 13 years, for example. Well, it's now known that, well, if you

look at the metabolites, they can be in circulation with a bioavailability of up to 48 hours, because they're continually recycled. And so, that now starts to then provide more of a plausible explanation for potential chronic benefits. So I think the first point is that their metabolism is unique and reflects the fact that they are treated by the body as xenobiotics. And then, of course, from there, we can start to maybe discuss the actual pathways and mechanisms through which these metabolites start to actually exert effects in the body.

DANNY LENNON: Yeah, and that's incredibly important. So maybe just to recap over that, these are treated differently to the vitamins and minerals, for example, in terms of metabolism that are seen as these different types of compounds that are then going to be metabolized directly at the liver after absorption from the small intestine, and there's a huge amount of metabolites then created. So yeah, just even from a point of interest about what the body does with certain compounds, it's interesting to consider polyphenols before looking at anything else. That's kind of cool.

ALAN FLANAGAN: Yeah, I think they're just fascinating, like, it's just really cool, the way that this potential for these non-nutritive – you mentioned at the start this idea that, oh, are they not essential; and, of course, from certain dietary circles, you might hear that emphasized as well we don't need them, but Gary Williamson at the University of Leeds, remember had a really nice paper where he described them as lifespan is essential. So if they're not essential in the way that essential fatty acids are, or essential amino acids or even indeed vitamins which are required for life, they, because of their action, can be essential to health span within a lifetime. And so, I think that's a cool way of conceptualizing them. So no, they're not nutrients, but they have this really cool metabolism, and that generates these multiplicity of compounds that do have biological activity, and the effect of that

biological activity, which is numerous, maybe to really enhance health span.

DANNY LENNON: Yeah, and I think sometimes people use that, something not being an essential nutrient as a kind of really lazy way of saying that, therefore, you don't need to worry about consuming it; in the same way people talk about carbohydrates aren't essential for survival or fiber.

ALAN FLANAGAN: Fiber, yeah.

DANNY LENNON: Right. But it's like, that doesn't necessarily mean it doesn't have a really good health benefit. And you see the same type of rhetoric here, an essential nutrient just has a specific meaning, it doesn't mean that you don't need things that are non-essential; it just means that, yeah, you don't have acute disorder that you're going to develop from a flavonoid deficiency in the way that if you develop scurvy or rickets, if you have vitamin C or vitamin D deficiencies. But that doesn't mean that we can't have better health from consuming them or that there's going to be negative health consequences in the long term. And I'm sure this is a point that we'll maybe circle back to later on.

ALAN FLANAGAN: Yeah.

DANNY LENNON: Okay. So with that set up, that's really useful to start taking a look at some specifics. We have a number of interventions that we want to walk through, but before that, it might be useful to look at a couple of cohorts. Maybe the first one to take a look at is the Kesse-Guyot paper 2012. This was a 13-year follow up looking at total polyphenol intake as well as the intakes of various subclasses of those. This is a kind of interesting paper for that reason, because it looks at both of them, main things looking at here is the total intake of polyphenols as well as those subclasses on things like language and verbal memory and executive function.

ALAN FLANAGAN: I think one thing that is just worth mentioning in all of this, because it's applicable to both the RCTs, and to, well, really any intervention that we might look at is this idea of cognitive testing in research. It's very challenging from a research perspective. There's different tests that can be used, and, of course, then with any measurement instruments you need to consider validity, and sensitivity, how does it correctly show an actual effect, and it's influenced by a lot of different factors like baseline age or health, cognitive health, both of which are often related. And then, there's external factors that could influence performance on a cognitive test, like, how well you've slept or mood or even there's habitual effects as in when people simply get better because of practice. So I think that's a rabbit hole in itself, but I think for people generally, just to kind of bear in mind that we're talking about different effects on say, for example, global cognitive function, which will be the composite of how someone has performed on a range of tests. And then, different tests can assess specific domains of cognition, like, episodic memory or verbal fluency or reaction time, and these are all proxies for different aspects of cognitive function. And it is important, because different studies using different flavonoids, or examining different kinds of polyphenol types, might often use different test types. So what I think with the Kesse-Guyot papers is interesting in the findings, the primary benefit was for episodic memory, but not executive function. And so, there's a granularity to the outcomes that's always important to bear in mind, when we're talking about the broad term of cognition or cognitive function.

DANNY LENNON: Yeah, and that's going to be important when trying to, I suppose, reconcile a lot of the data in this area where, like you said, you can look at different studies, and it may at first then seem, well, this is just a complete mess, it's just kind of inconclusive, because some seem to be positive, some seem to show no effect what's

going on. Well, there's a number of levels that we need to look at, one is what is the actual outcome being measured, which you just mentioned there. Second is what subtype of these compounds are we looking at. And then third we'll get to is, well, what is the dose or other aspects related to the specific paper. And again, this is just another way to start kind of training in that ability to not only interpret a specific paper, but then reconcile that with the other ones that you're reading rather than it becomes so overwhelming because we're seeing different measures or seemingly conflicting results in an area, as opposed to how do we work through this to come to a conclusion.

ALAN FLANAGAN: Yeah.

DANNY LENNON: So just to clarify for people, when we talk about this as a kind of 13-year follow-up, they initially collected data from a number of 24-hour diet records, a minimum of six that was collected in a kind of two-year period in the mid-90s; and then 13 years later, we have this follow-up, and they used those dietary records and referenced them with that database that I mentioned earlier, that phenol-explorer database, to kind of get an estimate of these intakes of polyphenols, and then making these comparisons of who's consuming the most over that time span, looked at this, in this case, this particular measure where we're looking at language and verbal memory, episodic memory, and then, executive functioning. So that was one of the cohorts that we wanted to mention.

ALAN FLANAGAN: Yeah, I think there's kind of two more that, I guess, set the scene. One was an analysis of the Nurses' Health study in the US, and that specifically from a food based perspective, found that high intake of blueberries and strawberries, but then also in looking at flavonoids, the high total flavonoid intake, and was associated with a delay in cognitive aging, equivalent to two and a half years. So what does that mean? That means essentially that 75

year olds in the study had cognitive test scores that were similar to 73 year olds. And so, this was again, showing this kind of effect on aspects of cognitive function in a way that appears protective. And then, you also had some of the analysis in the US, the Chicago Health and Aging Project did look at specific flavonoid subclasses, and found that particular types of anthocyanins were associated with significantly lower risk of Alzheimer's and dementia at the higher intake compared to the lower. So we have a number of cohorts, the one you mentioned, obviously, was in France; there was another one from France, which was the Three-City Study; and then there's the couple of cohorts in the US; and they've all tended to point in the direction of higher versus lower intake, having either a benefit on preserving cognition or some domain of cognitive function, particularly memory, and also being associated in terms of outcomes with lower incidence of dementia and Alzheimer's in particular.

And those studies, again, in terms of considering “warts-and-all” so to speak, those studies are often conducted in populations that are well over 60 at baseline. There are some other factors to consider with that particular outcome, such as fish consumption, and it's important that those dietary variables are controlled for, and the better controlled analyses have accounted for that. But that in and of itself brought the question of, okay, well if we're talking about a potential benefit in elderly people, would we still see this benefit in younger people or people that are otherwise kind of not at risk of some degree of kind of cognitive decline or attenuation. And, of course, we'll discuss the fact that there is that evidence, particularly in some nice intervention studies.

DANNY LENNON: Yeah. One of the other cohort was a group of Japanese Americans in Washington State where they were looking at fruit and vegetable juice intake, and compared from the highest

was people who consumed at three or more times per week, versus people who consumed at less than once per week on average, and that was like a seven to 10 year follow-up on average. And that was looking at Alzheimer's disease risk, and you had a hazard ratio of 0.24 for those consuming the most relative to those who drank the least of those drinks. But interestingly, when you look at that kind of, in between, so people that had an average of one to two, like fruit and vegetable juices per week, you saw a slight risk reduction, so a 0.8 for hazard ratio here. But if you look at the confidence intervals, just this massive range where you can already make any conclusion, so again, it kind of ties back to a thing that we often reference in relation to looking at say the impact of fruit and vegetable intake on a whole range of outcomes, is that the threshold that we're looking at, and the dose becomes very important, that sometimes in small changes, it looks like there's going to be no benefit. And it's only when you get to a higher amount or a very high intake that you actually start to see, oh there is a clear benefit to getting enough of these. So yeah, maybe consuming one or two extra high polyphenol drinks per week isn't going to do much, but if you're someone who's doing that every day, you're starting to push into these different ranges perhaps.

ALAN FLANAGAN: Yeah, absolutely. That contrast in the dose, and I think for a lot of the studies that have found a benefit, the contrast in the exposure for say, flavonoids, can be quite large. I mean, if I remember from the Three-City Study, we're talking about kind of over 400 milligrams compared to kind of less than maybe 50. So you're seeing that with, whereas, yes, if you're comparing within this narrow range, it may be more difficult to see an effect; and it doesn't mean there's no effect of that exposure, it just might mean that people in that reference category simply aren't consuming enough. And this research, I think it's always the one that I think for the prospective cohorts, does meet a degree of cognitive dissonance. And to a

degree, it's understandable, like, Alzheimer's is a condition for which there has been literally no pharmaceutical breakthrough. I think only one or maybe no drugs have been approved since 2003, and so, when you come along with a condition like that, and then say that blueberries might reduce risk, I can understand that people just kind of look at you and go, no, it's just like, again, we're talking about the relativity of the risk comparing higher versus lower intakes, no one's saying that blueberries are a cure for a disease, for which there's been no pharmaceutical breakthrough.

I think this might be a good point to maybe discuss some of the potential mechanisms that have been identified, because we've – okay, so we've said that there's these long term associations that are quite consistent depend, you know, in different cohorts, different populations. And they're having these beneficial outcomes in terms of neurological health, brain health. So the question then becomes how, and so, I think this will give us some context then to discussing some of the interventions. The historic thinking, so to speak, with these compounds was simply antioxidant activity – oh, they're rich in antioxidants, that must be the benefit. It's not believed now that antioxidant's action is what accounts for their main effect. And there's also little evidence that the compounds cross the brain barrier in significant concentrations, particularly, the parent compounds. So the question then becomes, well, how do they act. Okay, so going back to that point we made about the metabolites, right? So we get this effect of these metabolites, that are then absorbed into circulation have a much longer time in circulation, and they're much more abundant and bioavailable in circulation.

So what happens is they actually act, and this is crucial to their mechanism of action. At low physiological concentrations, you don't need enormous doses of this to have an impact on the brain. There's three kind of main pathways

– well, three is really, really grossly limiting – there's two broad, shall we say, outcomes that tend to be influenced. One is kind of a prevention side, where it impacts multiple pathways that influence things like neuroinflammation, and neuronal viability, so protecting neuronal survival, for example, or the inhibition of apoptosis. So these are these protective mechanisms, prevention of neurodegeneration, and protection against brain aging. And then we could flip over to kind of positive effects and, again, multiple different pathways where they influence things like memory and cognition, and that might be through enhanced cerebrovascular blood flow, and it might be through influencing pathways like brain derived neurotrophic factor. And these are important to the potential chronic effect, because what's known about activation of BDNF pathways, it's not an acute response. So that's more likely to be something that is influenced chronically over time. So they're kind of broadly speaking, I mean, we could start getting into, like, all of the kind of actual discussion of the actual pathways themselves. But I think, again, that would just kind of blind people with excess terminology. So I think suffice it to say that the main kind of mechanisms of action are on protection of the brain, basically, in terms of protection against inflammation, and then also protection of stimulation processes like neurogenesis. And then, there's also the kind of enhancement of memory and cognition through factors like increased blood flow to the brain, synaptic plasticity and otherwise. So there are very well kind of described mechanisms at this point, and that's not to say there may be more to uncover, but broadly speaking, we could consider it as inhibition on one side of negative processes and activation of positive processes on the other side.

DANNY LENNON: So yeah, that's really useful to set the stage for, and, I agree, we don't need to necessarily dive through all the pathways for this particular conversation, let's get into some of the

intervention trials, because I think that might bring some clarity to the situation, because it's great to look at cohorts and to look at things like a polyphenol rich diet, but to actually really refine down to see what we actually know, is having a benefit, we can learn a lot, I think, from some of these specific trials. So I think, as we kind of had discussed previously, a nice way to look at would be to maybe take some of these different groups, and work through some of the trials that are maybe quite illuminating to people. If we start off with flavonoids, and, in particular, one food type that has been looked at as being things like blueberries or blueberry based drinks, I know you've discussed before the Barfoot et al. trial, do you maybe want to just give a quick introduction to people about that trial, what we're trying to assess, and why we're bringing that up?

ALAN FLANAGAN: So these are interesting trials, because in some ways as well, they're great kind of illustrative examples of some of the challenges that nutrition research faces in terms of placebos and blinding and these kinds of issues. But this was interesting, because we discussed cohorts where we're largely looking at populations that are sometimes over 70 in terms of when the analysis is being conducted. So question bags then, well, might we see effects in other life stages. This trial was looking at seven to 10-year-old schoolchildren. It used a wild blueberry powder, which was concentrated, but it was equivalent to about 240 grams of fresh blueberries. And it also contained a specific amount of anthocyanins in the region of 250 milligrams of anthocyanins. And just for people listening, the relevance of when we're talking about the food and the compound, is the fact that what these compounds are reflecting is pigmentation. So anthocyanins tend to be found in purple or kind of dark purple type foods, grapes or blueberries. So, the actual chemicals themselves are giving the pigment to the food, which is why strawberries, which are red, contain different types of flavonoids to say blueberries.

So we're talking about this blueberry powder, but equivalent to 240 grams of fresh blueberries, a dose of 250 milligrams of anthocyanins. And what was good about this trial was that some other studies, one of which we'll look at, have often not matched the placebo and the intervention if they're using a drink based intervention for glucose or fructose, for sugar essentially. And we know that this is, you know, sugar is something that can sustain cognitive output and, in and of itself, have a kind of impact on some cognitive parameters. So this managed to make sure that it matched not just glucose and fructose but also vitamin C to the content that was in the intervention blueberry drink. And they also gave the participants both the placebo and the intervention drink and these kind of opaque flasks. So they couldn't tell the difference, so the actual participant was blind to the treatment. And they had a number of cognitive tests to look at things like attention, sustained attention for – basically vigilance, attention-vigilance, efficiency of reading, and a couple of other tests. What was interesting about this trial was it was looking at acute effects over a six-hour period, really reflecting a school day.

The intervention and placebo were consumed in the morning, and the children were tested over six hours on the actual test day itself. And so, this was then looking really at some acute effects, like, we've discussed, cohort studies so far, which we're talking about chronic effects. So question bags, and this, again, in the context of previously question mark over whether they could, if there was this very short half-life, how could you get effects even six hours later. And what was found in this study was that they had quicker reaction times in some of the cognitive testing, and enhanced verbal memory performance on one of the other cognitive tests, so they recalled more words than the placebo group. And so, in this intervention in school children, this effect of a anthocyanin rich or flavonoid rich, but specifically

anthocyanins in this case, which would again in a real life context, in a food based context, equate to about 240 grams of fresh blueberries showed a benefit on acute cognitive performance in seven to 10-year-old children. So it was coming really to the other end of the life stage at which the prior observational research had largely been conducted and demonstrating a benefit, even in an acute context.

DANNY LENNON: And so, within this, to kind of link back to some of those previous mechanisms that you'd mentioned, because, of course, if we're looking at something like disease risk reduction, and we know that there's kind of implication around neuroinflammation there, so that kind of mechanism of protection in relation to polyphenols seems important there. For something like reaction time or some of these cognitive improvements we may see in young children, do we know kind of why that was the case in this particular issue, or is that still an open question?

ALAN FLANAGAN: So from some of the wider research and, again, coming back to the mechanisms, it appears that one of the main drivers of acute benefits is an improvement in cerebrovascular blood flow, and that appears because pathways like brain derived neurotrophic factor, BDNF, would not be expected to be something that is activated in a kind of immediate sense necessarily. And so, that might explain chronic benefits, but it appears that the short term effects may be mediated by enhanced cerebrovascular blood flow. So basically, more blood flow to the brain corresponding with kind of better cognitive performance on different acute tasks, particularly as they seem to relate to things like reaction time or a kind of more immediate recall based cognitive battery test.

DANNY LENNON: That is looking at this blueberry drink in children to maybe kind of keep within this kind of same general group, and if we're looking at flavonoids, and again, if we keep looking at

berries, one of the trials that you outlined was a paper from Whyte and colleagues, 2019, which in this case, was looking at young healthy adults as opposed to children, and this time using mixed berries as a source of flavonoids. Again, can you maybe give us an introduction into some of the general setup of this particular trial?

ALAN FLANAGAN: Yeah. So it's again from the same research group or involved in both. So this was a similar enough design in terms of randomized placebo controlled and single blind participants blinded intervention, but this was looking at a mixed berry flavonoid rich intervention on cognitive function, again, over six hours. So a similar testing regimen, and a design to the study we've just mentioned in terms of the seven to 10-year-old school children, 20 participants in each group, and they were young and otherwise healthy, so I think there were 23 in terms of mean age, and they follow the low flavonoid diet for 24 hours prior to the test day, and then a 12-hour fast proceeding the actual baseline and the test battery. So that was conducted – the baseline testing was conducted when they were fasted at 9:00 a.m. And then afterwards, they consumed this flavonoid rich smoothie, and it contained overall a total polyphenol content of 14 grams, within that anthocyanins similar to the last intervention about 250 milligrams, and proanthocyanidins 256 milligrams, and there was some flavan-3-ols and flavonols, which are other components – we're actually going to come back to some of them.

So, basically this is an anthocyanin and proanthocyanidin rich flavonoid compounds. So the total flavonoid content, I said the total polyphenol content, 14 grams total flavonoid content was 570 milligrams. So they drank that after the battery, the baseline fasted battery, and then cognitive testing, then an hour and a half, three and a half and six hours, then leisure, and the cognitive tests lasted 30 minutes. So similar tests used in terms of the

modified attention task, and they also looked at task switching, which is designed to measure mental flexibility. So you have to identify displayed numbers in segments and identify which is an odd number, which is an even number, and then you've got to switch, and what they found was that the kind of modified attention, again, similar to the prior study, were in the intervention group, they were more accurate in their responses. And what was interesting was that what really drove the difference between the intervention group was that, and the placebo group was that in the intervention group, it was that their performance was sustained in this task over the six hours. While in the placebo group, they did, if you were to look at it, looks like at the two-hour mark, they're kind of still similar to the intervention group, but then they drop off between two and six hours. So what you were seeing was essentially an acute effect on preserving cognition in response to these modified attention tasks over the course of this six-hour period.

And again, this was one where the investigators were, and this is, again, quite crucial, matching the placebo drink for the sugar content, the total carbohydrate content, and the vitamin C content of the intervention drinks such that the primary difference between the intervention and placebo was the content of flavonoids and polyphenols. So what this was really showing was a preservation of cognition, not necessarily an improvement of cognition. But it is important when we're trying to marry up, I think the kind of biological or mechanistic plausibility, because what has been shown with anthocyanins is that the improvements in factors like, say, memory or recall, primarily occur at about 90 minutes post ingestion, and about six hours post ingestion. And this corresponds to peak concentrations of anthocyanin bioavailability, and those times also correspond to peak increases in cerebrovascular blood flow. So, there's a kind of a converging of different plausible

explanations which, although the magnitude of these differences in these tests can be small, I think it's still providing us with a potential, again, a plausibility to certainly some of the long term effects; but, in particular, this marrying of the change in cerebrovascular blood flow corresponding with peak anthocyanin circulatory bioavailability corresponding then to observed differences between a treatment and a placebo group.

DANNY LENNON: We're starting to see this across a number of these different groups, okay, so similar types of setup, there might be differences in exactly what type of food or beverage was used, and slight differences in methodology, but we have a bit more confidence as we start to see this emerging in different demographics, which is what we've seen so far with these first two. One of the other papers to maybe mention because it is similar in this respect is 2019 Haskell-Ramsay et al. paper, which use this grape juice. Again, this was looking at young adults similar to Whyte, but was using a grape juice here, so a purple grape juice specifically; and that this was interesting in the fact that they used this commercially available grape juice containing mainly Concord grapes which we mentioned a bit earlier, and they seemed to have this highest concentration of polyphenols relative to other types of fruit juices that are usually available. And there's also this mix of different compounds that they have within them, the flavan-3-ols that you mentioned, but also anthocyanins which make up a good chunk of that. And so, in terms of the dose, I think in this particular grape juice was anthocyanin content of 138 milligrams per liter, and the total phenolic content was 1500 micrograms per milliliter. So we have this purple grape juice used 230 milliliters of this juice versus a sugar matched control, again, which you noted in the other trial, healthy young adults, and I think the main findings of this particular trial was an improved reaction time using grape juice this time as the exposure, as opposed to

the mixed berries that we mentioned in the previous study.

ALAN FLANAGAN: Yeah, and that, again, was kind of corroborating the previous, the effects being evident in otherwise healthy young adults, which again, is important when we're thinking about the fact that cohort studies primarily look at elderly populations. And it's illustrative of the fact that there is a broad range of polyphenol rich foods or, in this case, if we're thinking about anthocyanins and that kind of particular pigmentation, it's not necessarily confined to, well, it must be blueberries, for example, because it's the actual flavonoid compounds themselves that are the exposure of interest; although the food as an exposure has relevance because some foods might have greater concentrations of particular flavonoid types than others; but really, it's illustrative of the fact that the exposure here is the actual flavonoid type. I think to kind of round out... because it also used Concord grape juice was, and this is an older trial, and it's goes back to 2009, but also used Concord grape supplementation, but what's this trial set apart from the previous ones we've talked about is because it was in elderly adults with mild cognitive impairment. So it was Krikorian and colleagues, and it was published in the British Journal of Nutrition. And it was a consumption of RCT with Concord grape juice for a 12-week period being consumed, again, with cognitive testing on the back end, and this was, give or take, between, I think about 4 to 500 milligrams of top flavonoids.

Now, what was interesting with this study is, yes, there was an effect, but also the factors that the placebo wasn't matched for sugar between the two intervention and placebo. So now, would that explain the entirety of the benefit? Perhaps, but it is a methodological kind of shortcoming that does have a little bit of an asterisk next to the study. But it still is interesting, because there was, you know, because of the population being elderly adults

with a degree of mild cognitive impairment. But again, the important differentiation from the prior studies as well, is that this was 12 weeks of daily consumption, ultimately leading to improvements primarily in kind of verbal learning, and verbal outputs, but kind of less magnitude of effect on other cognitive benefits. But I think that, even with one or two of its methodological shortcomings is still an interesting intervention when we consider the cohort studies And like you said, the Washington Study in particular, showing more regular consumption of this, of say, polyphenol or flavonoid rich vegetables and fruits in a kind of daily context, whereas – and this was a three-month study as opposed to just an acute testing intervention.

DANNY LENNON: Yeah, and so, based on those few studies that we've just mentioned here, what we can then start to conclude is that if we look at polyphenols, and, in this sense, anthocyanins really, particularly a focus from blueberries or from Concord grapes as a food or a juice, depending on the study we're looking at, that we're seeing a variety of benefits related to cognitive function generally, and some of the specific outcomes are different between those studies; but we're seeing improvements there, but what is, I suppose, interesting or certainly that can give us a bit more confidence in some of these results is that we are seeing it in those different groups. So that first study you mentioned from being in young children, then we see it in adults, then we see it all the way to elderly adults with mild cognitive impairment as well. So across that whole span, we're seeing a variety of different improvements, which should probably give us a bit more confidence relative to seeing one specific trial.

ALAN FLANAGAN: Yeah, exactly. And I think when a common knock on nutrition interventions, particularly like this, look, these are small trials, they're not highly powered, we are talking about quite fine differences in terms of some of these outcomes, but the relevant context that we have to

package this back up in terms of if we have seen this effect across the lifespan in both acute and more long, well, three months is not long term, but acute and more, shall we say, kind of intermediate term timeframes, well, then the question then becomes, well, what would be the effect of this over the course of a lifespan. And at that point there is a reasonable inference, if there are some of these effects in short term contexts, and indeed over kind of medium term, that you might have this kind of additive benefit over time, if this was a characteristic of diet sustained over the course of kind of adolescence and adulthood.

DANNY LENNON: Right. And then that's where we can start bringing in some of those long term cohorts and looking at studies where we're looking at polyphenol rich dietary patterns, for example, and if we're starting to see that marry up with these shorter and intermediate term trials, and also with some of the mechanisms that we've discussed, then, again, that's the view which we need to answer any question is through looking at all of those different lenses at the one time. Cool. So that's looking at some of the anthocyanins specific stuff. Maybe to look at maybe this next subgroup of flavonoids would be to looking at some of the flavonols that are specific in cocoa and chocolate. One of the studies you outlined to me was the Field et al. paper 2011, why is this one that you particularly like to bring up?

ALAN FLANAGAN: I think it starts to show the diversity of the compounds themselves, so for this now, we are talking about – so we've just been talking about obviously, anthocyanins or proanthocyanidins as two types of flavonoids, but now we're talking about flavanols, so F-L-A-V-A-N-O-L-S, cocoa flavanols rich in this flavonoid subtype, and we're also seeing effects on different parameters of cognitive function. So this was an interesting intervention which compared a dark chocolate containing at the equivalent of, well, containing 720 milligrams of cocoa flavanols. If I recall the actual dose for dark

chocolate would be that this would be say for an 85% cocoa dark chocolate, that would equate to about, I think, 40 grams, give or take. But so 720 milligrams, and then the placebo is white chocolate, because it doesn't contain any of these compounds. And it was really looking at, again, a kind of an acute effect, and there was a kind of a one-week interval, so they're crossed over. So the intervention group consumed – well, all participants consumed the intervention, the dark chocolate, and then the white chocolate placebo or control group with a week in between. And I think the field study in particular is interesting because, what you see with that study was an increase in visual acuity and visual spatial working memory. So these are kind of different cognitive domains that are being assessed, and it's obviously a different chemical compound in terms of the flavonoid that's being looked at from the previous interventions, and spatial memory and, again, reaction time improved in some of these domains as well.

So, this was again an acute study, really, what was interesting with this was the primary outcome again being visual function, broadly speaking, a number of parameters, visual acuity. But this also appeared to correspond to increased cerebrovascular or cerebral blood flow and specifically retinal blood flow. So that mechanism, even though we are now talking about a different flavonoid subtype, and even though we are talking about different parameters of cognitive function that have been tested, there seems to be a unifying mechanism in terms of the enhancement of blood flow to the brain.

DANNY LENNON: Super cool. So one of the studies that I think might be useful to bring up at this point as a kind of good teaching point for us in relation to interpreting and reconciling evidence is a paper out of Finland, Suominen et al., 2020, which also looked at dark chocolate flavanols. In this particular study, their intervention group was receiving 50 grams of dark chocolate, and that

contained 410 milligrams of flavanols per day as the intervention. And again, this was looking at cognitive function, but this particular trial resulted in there being no differences between the groups, so it seemed to indicate there was no real benefit or effect of this higher flavonol intake relative to the control group. Now, why this is useful to bring up is because there's, again, if someone first sees this, we don't want the immediate jumping to the conclusion of, oh well, this is a last cause, how we can reconcile this stuff. And so, we have to think through, well, what does this actually mean. So in relation to this being seemingly conflicting with the field study, what would be the first few things that that came to your mind?

ALAN FLANAGAN: The most immediate thing was the actual dose, so forgetting the actual equivalent in food based terms, in terms of dark chocolates, 410 milligrams of cocoa flavanols is a lot lower than the interventions that have found the benefit. So the trial that we just mentioned, the Field and colleagues trial, like we said, had 720 milligram intervention of cocoa flavonols. There was also a study by Scholey and colleagues, Crystal Haskell's group again in 2010, that actually looked at two levels of cocoa flavanols, 520 milligrams. So this was in a drink form, and 995 milligrams, again a matched control acute study, visual acuity aspects of visual cognitive function. But also things like mental fatigue were assessed in this study over different time points and the cocoa flavanols both levels of intervention reduced subjective mental fatigue, where they kind of administered these battery of cognitive tests two minutes apart, designed to kind of wear out the brain a bit. What could also be a factor that we need to bear in mind is that, particularly this trial, and also the previous trial that we mentioned, the Field study, they were trials in otherwise healthy young adults, the Finnish trial, the Floseco trial was in healthy, cognitively healthy older adults, but 65 to 75 years of age. So it could be an interaction of dose, and it could be an interaction between

dose and age, so those are both important factors. But certainly, the most striking seems to be that there's an argument based on previous research that this was simply too low a dose perhaps, and what would have been the effective – because they compared 410 milligrams to dark chocolate containing only 86 milligrams; if they'd had a third group consuming, say, I don't know, 800 milligrams, might there have been an effect. So I wouldn't be writing off cocoa flavanols based on this study, simply because of the potential for the study to have been under dosed, but also aware as well that previous studies have been in younger adults, which may be relevant.

DANNY LENNON: And again, this is just another useful point for us to think through when we're starting to interpret studies, again, along those lines of not only looking at dose, but why might that be the case, is there a certain threshold we need to pass in order for there to be benefit, or is it simply that we need to have a sufficient exposure contrast when we are trying to detect a difference, and that might shed some light on why there may be seemingly conflicting results. So I think that's just useful to bear in mind, but there is indeed a number of studies which suggest this benefit to cocoa flavanols – if we maybe want to start rounding this out with some of the citrus related studies perhaps?

ALAN FLANAGAN: Yeah, so again, for listeners, we're now swapping the A in cocoa flavanols with an O for citrus flavonols so these are F-L-A-V-O-N-O-L-S, and there're compounds people may have heard of like hesperetin or narirutin, and these are interesting compounds themselves in terms of potential mechanisms of action, in terms of the brain – some of these pathways we discussed earlier in terms of enhanced learning and memory pathways. But what's interesting is that unlike some of the trials that have used anthocyanins, where factors like executive function have not been increased, even though things like reaction time or verbal recall episodic memory maybe, the citrus flavanols in

the Keene study were shown to result in increases in global cognitive function, while there's another study Al Harvey and colleagues which showed increases in assessments of executive function. So again, it's highlighting that the actual precise effects may somewhat differ, because the compounds themselves structurally differ and may act through different pathways. The Keene and colleagues study was looking at a high flavanone citrus flavanone versus a low flavanone drink. The high drink, the kind of main intervention drink contains 550 milligrams of hesperetin per liter, and then 60 milligrams of this other compound known as narirutin with a daily 500 milliliter serving that was about 300 milligrams of total citrus flavanones, and then the low drink only had 37. And these concentrations were natural to the actual product itself, so the participants in this study were consuming this citrus flavanone rich drink daily for eight weeks, their average age I think was 67, so they were healthy, older adults. And they also crossed over to the low flavanone, citrus flavanone group with a washout period of about four weeks in between. So global cognitive function was the primary outcome, and we could think of this as a composite endpoint encompassing the average scores on a number of different tests, again, interesting as it relates to the trial population. So we're kind of covering the lifespan, so to speak, with some of these interventions, and we're also distinguishing between interventions that look at more acute effects over the course of maybe an immediate day following a treatment or an exposure. And then, we're looking at the effects of consistent consumption of this over time, and this is, yeah, this is an interesting intervention for the population looked at, in terms of healthy older adults, and also the fact that it was over a kind of a longer period than some of the acute studies.

DANNY LENNON: Fantastic. Yeah, I'm conscious of time, and I did want to get to maybe mention chlorogenic acids, so we won't go into too much detail, but I

think this is worth bringing up because right at the outset, we said when we talk about this general class of polyphenols there are different subtypes, and there's kind of a couple of branches we mentioned at the outset was flavonoids with these many subtypes that we've just walked through. But on the other side, non-flavonoid compounds, we have these various other phenolic acids, one of those is chlorogenic acid. And why I think it might be of use bringing up now is because we can connect that back to some of our discussion in the coffee and health episode we did, which I'll refer back to in a moment. But these chlorogenic acids are probably the main polyphenol that is in coffee, and for those people who did listen to the episode, you will remember us discussing that some of the benefit we see in terms of some of the epidemiological data around coffee consumption is likely down to its polyphenol content in some degree, depending on the outcome we're looking at. Now one of those outcomes was things like Alzheimer's disease risk and dementia risk, where whilst that data wasn't necessarily as strong as what we're seeing with, say, Parkinson's disease, which we also looked at, but that seems to be more related to the caffeine content of coffee; with something like Alzheimer's and dementia, there is still a decent amount of that literature supporting this kind of J shaped curve of risk that kind of two to four cups of coffee per day exerts this potential protective benefit; and that may be down to the polyphenol content, particularly when we look at the average diet that someone in the general population has. We know the vast majority of people, for example, under consume fruit and vegetable intake relative to dietary guidelines, and therefore, if a lot of people do drink coffee in decent quantities, that leads to coffee being a significant contributor to their polyphenol intake, which they are not getting from fruit and veg. So with all that said, that's made chlorogenic acid something that's either been looked at in coffees with high chlorogenic acid

contents or even as an isolated compound extracted from coffee, there's a couple of intervention trials we could mention, but we probably don't have time, that have looked at this in relation to cognitive function. But I think it was just something that's worth bringing up, seeing as that might relate back to some of that discussion that we see around coffee, and particularly, as we discuss some of that epidemiological data of coffee and Alzheimer's and dementia.

ALAN FLANAGAN: Yeah, I think so, I mean, I think if I remember, so a cup of coffee, on average, can contain anywhere between, say, 50 and 100 milligrams of chlorogenic acid. And, of course, linking this back to some of the metabolism and bioavailability factors that we discussed earlier, this is something similar, where there's this metabolism of chlorogenic acid into kind of caffeic acid metabolites that are also absorbed and kind of have this effect. And, yeah, I think there's two interventions, in particular, that we were looking at that are both out of Japan, and seeing a kind of benefit to chlorogenic acid administered in an intervention context. So yeah, it kind of married up some of what might be one of the most, other than the caffeine content of coffee, important associations and biological plausibility aspects to that J shaped curve we see with coffee and neurological health outcomes.

DANNY LENNON: Excellent. Yeah. So given our time restriction here, let's start pulling this into some things to leave people with. I suppose, when we're looking at conclusions in this area, already any area, we probably have a few different buckets we could put stuff in; one would be what we actually know or have a sufficient amount of evidence to make a conclusion about; then we have these other areas that seem promising, but we still need more things to validate if they actually play out. And then we just have things that are generally unknown, and there's probably a lot of that in relation to polyphenols. So if we were to maybe pull in

those conclusions off stuff that is known or kind of well-established at this point, that relates to some of our conversations today, what would be those main conclusions when it comes to polyphenols, neurological health, cognitive cognition, etc.?

ALAN FLANAGAN: Yeah, I think, one, we could kind of broadly say that there is relatively consistent base of support from interventions, although the magnitude of effect may be small, relatively consistent base of support from intervention studies that would provide a plausible kind of explanation biological plausibility to some of the long term associations, in terms of beneficial effects on preserving cognition or, in fact, being protective against adverse neurological outcomes. The effect appears to be evident across the lifespan. It kind of gives some truth to Gary Williamson's terming of these as, “lifespan essential”, so from children to healthy young adults to elderly adults with mild cognitive impairment, there's this effect. There may be no need to necessarily separate out acute versus chronic effects, i.e., both are likely to be relevant, both are likely to be important in terms of acute effects, perhaps reflecting something like enhanced cerebrovascular blood flow, while more chronic effects, for example, influencing BDNF and those associated learning and memory processes over time; their extensive metabolism and the greater understanding of the activity of metabolites provides a degree of even further biological plausibility to why there might be, again, kind of more chronic benefits, rather than just looking at the parent compound's short half-life. And then I think, as is important with nutrition, when we're thinking about some sort of rubber hits the road for practical application, although we didn't discuss it today, some of the interventions have used things like compounds like resveratrol and supplemental doses that you would never get through diet, I think for exposures like blueberry anthocyanins, cocoa flavanols, citrus, flavanols or flavanones, these

are obtainable at levels of consumption that could be habitual in the daily diet, like we said, anywhere between 150 to 250 grams of blueberries provides anthocyanin doses in the range of what we've seen in interventions, the cocoa flavanols depending on the cocoa content of dark chocolate could be anywhere between 20 to kind of 50 grams, and the citrus flavanone interventions could be anywhere from 250 to 500 mil of orange juice, some of which have been commercially available. And this is only factoring in a number of specific foods. We still have rich polyphenol based sources of multiple different flavonoids from the wide array of different pigmented fruits and vegetables. So yeah, there are converging lines of evidence I think that we could make some reasonable conclusions that would point in the direction of certain food based recommendations, while having that caveat with nutrition that weighing up a benefit to harm analysis there is likely to be little harm for making these recommendations.

DANNY LENNON: As often fits in with this looking at higher intakes of fruit and vegetables tends to correlate to yet again benefit, and in this sense, it would be having a variety of different types of those on the basis that pigmentation that you mentioned. So this sometimes gets boiled down to eating across a rainbow I think is sometimes what you might hear a dietitian talk with a patient about it as a simple message of getting different types of colors of fruits and vegetables, given that that means they're going to have these different classes of compounds, as you mentioned a bit earlier. So yeah, that is I think, that was just to round it out there.