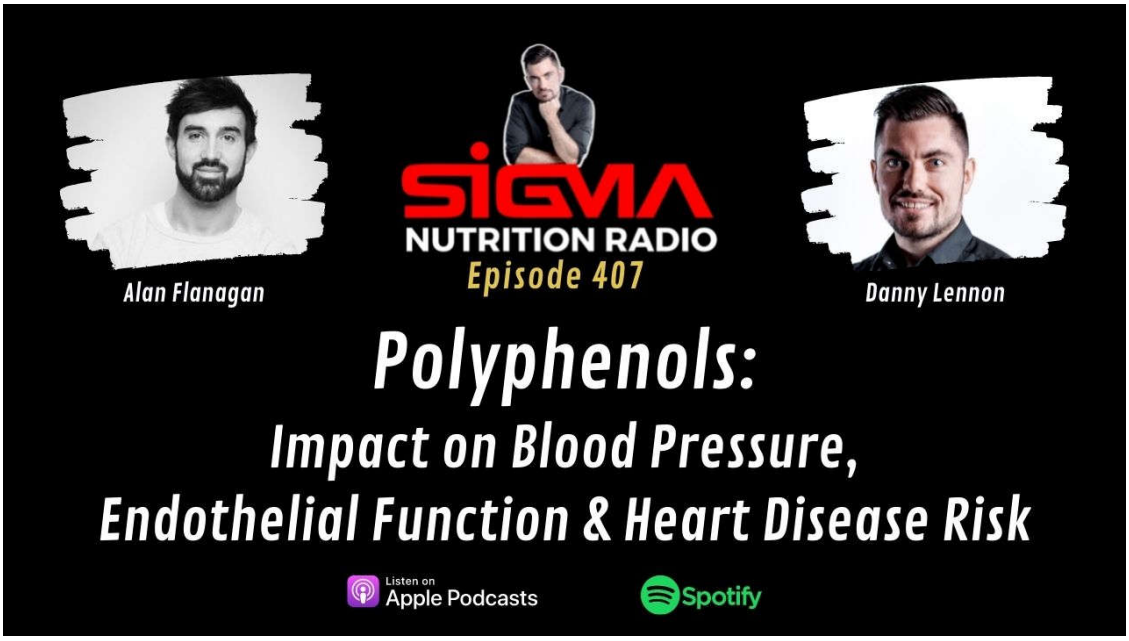
#407_ Polyphenols - Impact on Blood Pressure, Endothelial Function & Heart Disease Risk



DANNY LENNON: The last episode was on cognitive health. Today, we are looking at another area of interest with a lot of investigation, the role in cardiovascular disease risk. And they have been looked at in relation to cardiovascular disease quite a lot, because there may be a number of mechanisms, that we could theorize why they would have an impact. And so, maybe if we start, Alan, with walking through a few of these, what do you see as the primary mechanisms as to why we would even think about polyphenols in relation to cardiovascular disease?

ALAN FLANAGAN: Yeah, last week, we obviously talked a lot about kind of brain pathways and brain derived neurotrophic factor, and this kind of effects on processes that may influence learning and memory. But when we were discussing those pathways, we would have mentioned that one of the strong correlates of the increased activity of these pathways are factors like blood flow, cerebrovascular blood flow. And so, it is known that one of the mechanisms of actions of flavonoids is having vascular effects and beneficial effects on nitric oxide production and activity, and also, from a cardiovascular health perspective, things like endothelial

function, flow mediated dilation. One of the concepts we talked about when we were discussing mechanisms.

And the last one was this idea that, broadly speaking, we could think of their action as both inhibitory and activation. So inhibition and activation, and so, for a cardiovascular health perspective, on the inhibition side, we have inducible nitric oxide synthase or iNOS, and this isn't something that you want to be activated necessarily because excess or increased iNOS activity can produce excess nitric oxide. So nitric oxide is good, but it's one of those things we don't necessarily want excess off, and that can relate to impaired vascular function and even mediate inflammation, vascular inflammation. So flavonoids inhibit inducible nitric oxide synthase, and then on the activation side, they activate eNOS which is endothelial nitric oxide synthase, and that's a beneficial pathway associated with increased blood flow, increased angiogenesis, and some of these beneficial endothelial and vascular effects that we associate with flavonoids.

So as far as kind of particular vascular effects there is that, and then there is also some evidence of their potential to perhaps modify lipid metabolism, both kind of in the fasted state and potentially postprandial fat metabolism as well, although the mechanisms here are not necessarily that well known yet, there are suggestions that, well, do they perhaps inhibit fat absorption at the level of the gut or do they perhaps influence cholesterol and triglyceride transport in lipoproteins in the postprandial period, and perhaps also the fasting period as well. But those mechanisms, as far as lipid metabolism goes, and flavonoids are certainly – we're on a lot less solid ground with mechanistic understanding compared to the endothelial function, nitric oxide influences and the overall kind of effects on vascular function that flavonoids have and a range of flavonoids have. And one thing I find quite

interesting about the whole flavonoids and cardiovascular diseases, we tend to think because these are non-nutritive bioactive food components that have required a lot of more recent study to elucidate their mechanisms of action, understanding of their metabolites and the activity of their metabolites that we discussed in the previous episode.

If you go back to the Seven Countries Study, flavonoids were one of the main variables identified as associated with lower cardiovascular disease risk. If you look at the 25-year follow-up of the Seven Countries Study, the three main variables that were included, positive or negative, that were associated with heart disease were smoking saturated fat, both having a negative impact and flavonoids having a positive impact. So these have not crept up out of nowhere in terms of our understanding, it's that there would have been these associations even as early as something like the epidemiology of the Seven Countries Study, but we're obviously in a much better position now to understand their underlying mechanisms and to actually look at human interventions.

DANNY LENNON: Yeah, to me, one of the fascinating things, when I was looking at some of this stuff around plausible mechanisms is that, originally, it probably would have been an assumption that they're having this antioxidant benefit, right? Because that's one of the main things that we know that's going on, so with cardiovascular disease it's some degree of inflammation, and if there's this antioxidant capacity of something like polyphenols, then that can have this direct antioxidant activity. And I think there is something to suggest that there is this inhibition of those reactive oxygen species. But what it seems now is that all these other potential mechanisms are being more and more looked at as probably being primarily responsible, given that there is this low bioavailability of polyphenols in the first place. There's all this cool stuff happening with their

metabolism, and the metabolites are generated, and then we're starting to see impacts on endothelial function or nitric oxide production. Or even I think there's some stuff around like T cells and their adherence to endothelial and all this other stuff, where that seems to be more likely, what's the vast majority of the effect as opposed to just some direct antioxidant capacity, which is probably the first assumption that originally would have taken place.

ALAN FLANAGAN: Yeah, and probably not something that is a particular focus now, the antioxidants activity is unlikely to be just the explanation for their bioactivity, which is interesting; and not that they don't have a potential influence on factors like oxidative stress, but I think that that model or that concept of dietary antioxidants get eaten, and therefore, have this free radical mopping up kind of antioxidant effect in the body. I think we know that that's not really how it plays out. Even the compounds that do act as an antioxidant tend to do so via kind of upregulating endogenous antioxidant defense systems, rather than roaming around, as this kind of like free radical muck or something like that.

DANNY LENNON: Yeah, so essentially, we're seeing these metabolites of polyphenol compounds acting as like signaling molecules for various things that are then having some of these mechanistic effects that you've just outlined. And we'll probably circle back to mechanisms as they come up through specific examples and specific studies, depending on what actual outcome we are going to look at. So maybe we can just start diving right in if you think that's a good place to start looking at some specific studies. And so, as we did in the previous episode, to try and give a flavor, no pun intended, of what is potentially going on here, we can bring up some specific examples of studies and a number of randomized control trials that we have here that might be illuminating in some way. So I think maybe the first one that we

could mention is the paper from Sanchez Macarro and colleagues, which is a 2020 RCT on citrus flavonoids. And this was essentially a food supplement that was given as a kind of yellow powder, a combination of grapefruit, orange, and olive extracts versus a placebo powder. So this was an eight-week study, the participants were between 40 and 75 years old, we had a final study population of about 51 subjects in the active supplement group, 45 in the placebo group with a relatively even split of men and women, and the primary outcome in this study was reduction of cardiovascular disease risk, assessed by flow mediated dilation or FMD as a measure of endothelial function, essentially. So before we get to any of the results, is there anything that you wanted to outline about the study or even with regards to the results, why is this a particularly useful study to mention?

ALAN FLANAGAN: Yeah, I think the kind of main findings, the flow mediated dilation vasodilation, so it's kind of an improvement of vascular function as well that we are discussing, and vasodilation obviously associated with other factors like blood pressure, we do see that consistently, and so, in some of these interventions that we'll discuss with this study, the flow mediated dilation vasodilation was not kind of what jumped off the page, because it was consistent. But I actually found more interesting in this study was the impact on LDL and oxidized LDL cholesterol. There were significant differences in flow mediated vasodilation, like you said, and in blood pressure between the groups, which the two of them, there's another study that actually did a correlation analysis between these factors, and, as you might expect, they're quite strongly related. But I thought some of the research on the effects on kind of lipids, and lipoprotein metabolism is slightly inconsistent, or, at least there's some findings that are underwhelming. And so, with this we saw a nine milligram per deciliter on average reduction in LDL cholesterol, compared to no change in the placebo group, and that's a not

insignificant level of reduction. And with that though, perhaps even a more striking magnitude of effect was the reduction in oxidized LDL, which was reduced pretty substantially in the intervention group. And this is after kind of, as you said, eight weeks of supplementation. And I thought that was interesting, because again, coming back to the kind of mechanisms, there is some suggestion that flavonoids can protect against the effect of oxidized LDL cholesterol. And that is some of the analysis or some of the mechanistic speculation as to why there might be brain benefits acting through pathways that prevents cell death that would be caused by oxidized LDL cholesterol. And so, with this, obviously, focusing more on the cardiovascular side, but again, there's always strong relationships mechanistically, between the head and the heart, so to speak, I thought this was interesting, because the protection against LDL oxidation is obviously a really important aspect of the pathogenesis of atherosclerosis within the vasculature. So the idea that there could be that protection at that level was, for me probably the most interesting finding in this study.

DANNY LENNON: Yeah, I know one of the other findings that I think the authors apparently made note of, at least, in the discussion was around interleukin 6. So a marker of inflammation, which obviously has had some associations in, for example, risk of myocardial infarction, where we see chronically elevated levels of this being a marker of inflammation, but here, I think the reduction was close on 40% in this, which then theoretically could have a meaningful impact in the real world. What did you make of the findings around IL-6?

ALAN FLANAGAN: Yeah, I think because, obviously, there are various inflammatory markers that could be measures, C-reactive protein, various interleukins and TNF alpha and others, and kind of paying attention to some of the interesting conversations around the use of

these markers, and whether they're subject to kind of more variability or which is more predictive than others, and some of the Mendelian randomization studies have suggested that actually interleukin 6 is more strongly related perhaps in a more kind of causal, although I'm more hesistant to use that term now in relation to MR, but in that kind of more direct relationship manner as a marker of inflammation than even things like C-reactive protein. And certainly CRP seems to be subject to a lot of variability, and so, there is an argument that is framed sometimes that IL-6 actually might be a more, shall we say, robust and marker of inflammation as it relates to cardiovascular disease risk. And so, I found that particularly where the effect with CRP was negligible, the effect of TNF alpha was negligible, but we got this or saw this quite significant reduction in IL-6 that could be something that is clinically relevant.

DANNY LENNON: Very cool. Anything more that we should mention on that study before we move on?

ALAN FLANAGAN: Nope, not from that one that I can think.

DANNY LENNON: Great. Well, maybe next we might look at the proanthocyanidins study, the 2019 RCT of Japan, I think Odai and colleagues were the authors on this. So this was looking at a grape seed proanthocyanidin extract, so proanthocyanidins abundant in things like fruits, seeds and nuts, depending on ones we look at. This was a study looking at adults with pre-hypertension, so defined in this particular study, I think based on Japanese guidelines of a systolic blood pressure of 130 to 139 milligrams of mercury, and/or diastolic blood pressure of 85 to 89 milligrams of mercury. Participants were 40 to 64 years old, and they were consuming tablets of either a low dose or high dose of this grape seed proanthocyanidin extract or a placebo over a 12-week period with 10 people in each group. For the particular outcomes and results in this study, what

particularly jumped out at you as why this might be noteworthy to look at?

ALAN FLANAGAN: I think for me, it was the magnitude of effects on systolic blood pressure. Some of the other studies that have looked at otherwise healthy individuals have seen reductions in systolic blood pressure, but they have been in the order of say, 3 to 5 mercury millimolar. Whereas with this, if I remember the grape seed extract was in these pre-hypertensive individuals, over the 12-week periods, I think they saw like a 13 mercury millimolar reduction in systolic blood pressure. So potentially suggesting that actually in people with pre-hypertension, you get a larger magnitude of effect, although interestingly, in this study, what is generally a more consistent finding in the other research on flow mediated dilation or vasodilation, it did not change significantly. But there was this particularly substantial reduction in systolic blood pressure and it was dose dependent as well, which was also interesting. So if I remember it was the 400 milligram per day proanthocyanidin extracts at which that magnitude of effect was primarily observed.

DANNY LENNON: Yeah, and then in one of the kind of subanalyses of that, I think, for the diastolic blood pressure, it seems that that was significantly reduced when you look only at non-smoking participants. But again, that's relatively small sample size overall, but is still interesting nonetheless.

ALAN FLANAGAN: It was, and some of the epidemiology of flavonoid intake and cardiovascular disease or even antioxidant, starchy antioxidant intake has suggested that smoking status might modify some of the associations. But it was interesting in this that certainly the effect on diastolic blood pressure seemed to be primarily evident in the non-smokers.

DANNY LENNON: Awesome. So that is a couple of RCTs looking at these essentially extracts or food supplements. If we look at the substudy from

PREDIMED, so this was 2015 study Medina-Remón and colleagues were the authors on this – this was a sub study of the PREDIMED trial, which has been mentioned a number of times in this podcast, but I think a lot of our listeners are probably familiar with anyway. So substudy of 200 participants – remember, these are people at high cardiovascular disease risk. And the three diets within here that were being compared, as you may remember, would be a controlled diet of a low fat diet, and then two different types of Mediterranean diet, one, where there's extra supplementation of extra virgin olive oil, and then one with nuts – so the Mediterranean extra virgin olive oil, Mediterranean nuts, being the two different types of diets. So we're comparing, essentially, three diets in this case, as opposed to just the addition of a flavonoid supplement per se. So that Mediterranean diet with the extra virgin olive oil was where people got, I think, a liter of extra virgin olive oil per week. And then in the nuts group, they would get 30 grams of different types of nuts per day, so walnuts, almonds and hazelnuts to make up 30 grams per day. So we're looking at a group of between 55 and 80 years old, and there's obviously going to be differences then in some of the polyphenol content given that there are different polyphenol compounds found in extra virgin olive oil as well as in nuts. And indeed, we see that within this trial when you account for those in the total dietary intake of different foods that those people were consuming over the course of this trial. So within this, we're looking at a number of different things that we can maybe walk through, but what is the first main thing for you, Alan, that you took from looking at this analysis?

ALAN FLANAGAN: So interestingly, because of those differences in potential contributions to polyphenol and with nuts as well, you've got phytosterol content as well, which these are all factors that need to be considered, and what I thought was interesting was that, if you just looked at the kind of basic effects unadjusted for any additional factors, it

looked like the olive oil intervention group had much more significant reductions in blood pressure, in both systolic and diastolic blood pressure. And then, interestingly, then when they adjust for baseline blood pressure, change in plasma and nitric oxide, and then other factors like smoking or physical activity or antihypertensive use, then suddenly, the effect of nuts adjusted for those factors became a lot more substantial. I mean, in fact, the effect on diastolic blood pressure was greater in the nut group with those adjustments made. So in just a raw analysis, olive oil reduced systolic blood pressure by basically about 6.14, the nuts by 2.69, and then once you adjust for those factors, olive oil still like a reduction of like in systolic BP of 5.79, but the nuts was like 7.26. And again, that same pattern was observed when looking at diastolic blood pressure. I thought that was kind of interesting that once you started factoring in these other variables, then the effect of nuts became a lot more on a par with what you might expect from olive oil, because the olive oil finding you would look at and be like, okay, well, that is something that I could probably marry in my head with the rich polyphenol content of olive oil, but the nuts were not to be outdone once we factored in these other variables.

DANNY LENNON: Yeah, it was quite nice in that, here was a measure of total polyphenol excretion within the urine.

ALAN FLANAGAN: Yeah.

DANNY LENNON: And so, then you start to see this significant correlation then between when you look at the quartiles of change in that polyphenol excretion in the urine, and changes in plasma and nitric oxide. And then, if people go back to our episode on dietary nitrates and nitric oxide, and its impact around health there, they can then unsurprisingly, see then this connection between this increased plasma nitric oxide, and then this decrease that we're seeing in systolic and diastolic blood pressure. And so, we're able

to see that both of these Mediterranean style diets were able to increase that polyphenol excretion in the urine, increase plasma nitric oxide, and then lead to these decreases in blood pressure in this kind of really nice manner. And it seemed like the higher that change in urinary polyphenol excretion, the greater the change in plasma nitric oxide.

ALAN FLANAGAN: Yeah, absolutely, and the kind of the greater the change in blood pressure. So yeah, I think the measure of urinary polyphenol excretion was a kind of a nice addition to this one, whereas the other studies were relying on the dietary quantification of the polyphenol content, and saying, this is the polyphenol content of this intervention food or supplement, whereas with this, obviously, this was the PREDIMED trial that went over five years, I think this analysis was based on four years' follow-up. But again a relevant population, I think, 80% of the participants had hypertension, 68 years old, like, so high risk cardiovascular patients which were the population that were recruited for PREDIMED, and these are certainly kind of stronger effects that we would expect when you're adjusting for medication use and these other variables that you would have to account for in a high risk population, whereas some of the interventions we described earlier, you're talking about healthy young adults and magnitudes of change that are not necessarily huge, probably important over the course of a lifespan.

DANNY LENNON: So with that, where should we go next, what is the next trial you think we should jump to, because we have a few to pick from here?

ALAN FLANAGAN: I think the two papers from the Italian group, I think they – because these were looking at polyphenol rich and also omega-3 enrichment diets, but looking more specifically at postprandial fat metabolism, looking at cholesterol parameters, and also conducting a kind of robust test day where they were looking at postprandial lipoprotein metabolism and

triglyceride and cholesterol enrichment and lipoproteins and influenced by a polyphenol rich diet. That was the 29 P paper, Della Pepa was the lead author. The original publication was a 2014 paper Annuzzi and colleagues and the American Journal of Clinical Nutrition. So this was an interesting study, so they had a baseline running with dietary intake. They then matched diets in terms of a control, and then they had a high long chain omega-3, so EPA and DHA group, they had a high polyphenol group, and they had a high polyphenol and high omega-3 group. And the composition of the diets was largely well-balanced for macronutrients and otherwise, and so, for the intervention diet, the difference was either the long chain omega-3s or the polyphenols, and the polyphenol content of the diets was really high in terms of the, I think, nearly 3000 milligrams a day of polyphenol intake in the high polyphenol diet. And this was looked at over eight weeks, so these diets were followed over an eight-week period. And there was an effect of the high polyphenol diet on both fasting and postprandial lipids in this initial study.

But I think what was most interesting within that is when we think about fish oils and their effect on cardiovascular parameters, it's primarily their effect on reducing triglycerides that is of most interest, and kind of overall positive impact on postprandial fat circulation basically, kind of lower triglycerides, you've kind of lower postprandial remnants and all of this kind of stuff. But in this study although there was this reduction in triglycerides that occurred with the fish oil group, it was the high polyphenol crew that actually had more of an effect overall on factors like VLDL triglycerides. And so, it was an interesting outcome to see, in this context now, we're talking about small margins, but to see nevertheless, that this kind of very high polyphenol diet actually kind of outperformed the omega-3 fatty acids on some of these relevant factors like triglyceride and otherwise.

And then, the other kind of interesting thing about this study was they used more robust measures of oxidative stress, which are known as isoprostanes – isoprostanes are kind of more reliable actual measures of oxidative stress. And again, you had this kind of significant effect of polyphenols, but no effect of the omega-3 fatty acids, and we might not necessarily expect that, we would possibly expect more of the – as it relates to oxidative stress, more of an effect of the polyphenols. But nonetheless, this overall kind of analysis showing essentially a kind of an outperformance of this really high polyphenol diet versus high omega-3s, although you might get people kind of argue that omega-3 intake wasn't necessarily enormously high. So I think that was the first study, and then there's the second study, which was basically a secondary analysis of that initial trial. Within that trial they had done before and after test days, they had conducted a test meal and then had essentially locked us postprandial lipid metabolism over six hours after this high fat test meal. So they had done the high fat test meal before the intervention, eight weeks in the diet on each diet, and then the high fat test meal after the intervention.

And this was interesting, because they looked at both VLD1 and VLD2, but the main difference with the VLD1 and VLD2 is in their kind of size and triglyceride composition, VLD1 tends to be larger, VLD2 tends to be smaller and we would consider VLD2 to be more atherogenic in terms of its actual size and composition. But this study is an interesting one because it's difficult to kind of tease out whether there was a number of effects found that differed relative to like a protein subclass. So, high polyphenol diet reduced postprandial concentrations of VLD1, but no difference in VLD2, it increased the cholesterol carried in intermediate density lipoprotein; and then in low density lipoprotein, it had no effect on the cholesterol concentration within the

lipoprotein, but it did change the concentration of triglyceride which was higher in LDL after the high polyphenol diet. Now the authors were then like, well, this changes the cholesterol to triglyceride ratio and it's this, okay, and that's kind of "hammed up" up is this beneficial kind of outcome, but then you look at the effect on HDL and that was the absolute reverse of the effects on LDL. So that lowered the triglyceride content in HDL with no change in the cholesterol concentration within HDL.

And I remember just kind of reading through this and trying to piece it all together and be like, okay, like, these are, yes, they're statistically significant findings, and then just trying to think about it from a more kind of practical perspective in terms of what we know, and I couldn't help but think, are these findings actually really that – is there that much of a change that we're looking at here, or, are there kind of lots of these little changes that may just kind of cancel each other out. Ultimately, what we're concerned about with atherogenic lipoproteins is their capacity to get in, be retained in the artery wall and dump the cholesterol payload in that artery wall. So if it's not, and yes, triglycerides are a really important part of the overall picture of metabolism, but I kind of came away from this still with question marks over whether these are meaningful changes from a kind of postprandial lipid metabolism standpoint.

DANNY LENNON: Yeah, we may be seeing this slightly different ratio in the amount of triglyceride to cholesterol in some of these LDL particles, for example, but if we're not getting any real meaningful difference in the number of these LDL particles or Apo B containing particles, if we want to look at it even a bit more granularly, or, their ability to get stuck within the arterial wall, as you mentioned, then kind of how much does it does it matter...

ALAN FLANAGAN: Yeah.

DANNY LENNON: You know, are we focusing on things just because – are we essentially looking at something that might be a proxy for an issue, but then mistaking that for something that's actually going to be problematic, yeah, that's a really good point.

ALAN FLANAGAN: Yeah, exactly. And one of the things within that is this idea that polyphenols may affect lipid absorption, but they even make the point that there is no change in fasting Apo B 48 which are intestinally derived Apo B containing lipoproteins, so from the diet. And there was no change in the kind of Apo B 48 area under the curve for that postprandial for that six-hour period. So although there were a heap of findings from this study with each specific lipoprotein subclass, they were like, well, this happened with the VLD1, this happened with VLD2, this happened with IDL, this happened with LDL, this happened with HDL, stepping back from it, I'm not convinced that the findings were particularly impressive at all from a kind of modifying atherogenic dyslipidemia standpoint.

DANNY LENNON: Awesome. So we'll move on maybe to coffee and chlorogenic acid. That sounds good to you?

ALAN FLANAGAN: Sure.

DANNY LENNON: As a reminder for people, last week, we'd actually mentioned this, and we went into more detail within our coffee episode in particular, about some of the epidemiology where we note this essential J-shaped curve in relation to coffee intake and cardiovascular disease risk, cardiovascular mortality, i.e., that between this two to four cups kind of moderate intake of coffee, seemingly to be maximally productive, and then increasing risk as you go further and further either side of that. And as we discussed in that coffee episode, one of the things that we know about coffee is it has a relatively high polyphenol content, and particularly, we can look at certain phenolic acids like chlorogenic acid, which can be high in coffee. So we

discussed some of that epidemiology before, but here we have a randomized control trial, in fact, a couple of them that were done by Charlotte Mills and Jeremy Spencer, and the team at the University of Reading, where they looked at vascular function and they were particularly focused on the chlorogenic acids that are in coffee to see their impacts there. So within these RCTs, what are the first things you think are most important to point out or that jumped out to you with these couple of trials that they run?

ALAN FLANAGAN: I think the small trial nature... these are small studies, male only, I think that's always something to consider when we're talking about some of these interventions. But as far as some of the kind of positives, I think, with coffee or caffeine, it's really trying to isolate some of these polyphenol effects. And so, what was certainly a strength of the kind of design of this study because I think we discussed in relation to some of the brain health interventions that sometimes you get factors like the sugar content between an intervention and control aren't matched or other kinds of potential variables that could have an impact on cognition, particularly, acutely. And these were acute studies, but they were matched for caffeine, so differing in their content of chlorogenic acid, one that goes is kind of a low chlorogenic acid group of around 89 milligrams, and the other was around 300 milligrams of chlorogenic acid, and looking at flow mediated dilation, again, is this outcome that we see quite commonly used in these polyphenol interventions. And so, I think another concept we've talked about before is this idea of a biphasic dose response, and this is also known as a hormetic response or hormesis where you get this effect at low physiological concentrations that might be towards kind of – and then you get this kind of sweet spot in the middle, and then high physiological concentrations – and there's qualitatively different outcomes based on where on that, so to speak, J-shaped curve your

dose of your exposure and your outcome lies. And so, with this acute study there was this response in relation to flow mediated dilation, which was greater with the high chlorogenic acid intervention, and that was observed both at one-hour and five hours post coffee consumption. And so, actually, what was interesting was when we consider this biphasic dose response is that five hours, the magnitude was greater again, in relation to the high chlorogenic acid intervention than it was at one hour post intervention. So this kind of effect going way beyond just that initial kind of one-hour measure.

DANNY LENNON: Yeah, and, I guess, one of the things that I think you mentioned in our previous episode, again, is when thinking about some of these kind of, I suppose the impacts of the timing here, noting that we therefore, with coffee consumption in particular is usually something someone will have multiple exposures to, if they're a regular coffee consumer across the day. So again, and it's something that's on a chronic basis, as opposed to just a one-off consumption of coffee having a lasting impact on health per se, it's this chronic exposure done usually, sometimes once per day, but often more than that, in many people that do consume coffee.

ALAN FLANAGAN: Yeah, and the five-hour mark was interesting, because it's been kind of when the focus has come more on to the metabolites of a lot of these compounds, it seems to be that, give or take, anywhere between kind of five, six, seven hours is where you get peak bioavailability of the metabolites. And that tends to then correspond with these with a maximal response to that outcome that you might get. We've seen that with cerebrovascular blood flow studies using anthocyanin interventions, that the increase in cognition corresponds to the peak of anthocyanin and bioavailability, which also is when the kind of the largest magnitude of effect on cerebrovascular blood flow is observed. And again, with this, you're seeing a

kind of that similar pattern start to emerge in terms of the potential activity ongoing of these chlorogenic acid metabolites.

DANNY LENNON: Awesome. So that is our coffee and chlorogenic acid. If I'm keeping track here, the flavonoid rich apples and nitrate rich spinach trial is the one that we need to look at next?

ALAN FLANAGAN: Yeah.

DANNY LENNON: I think this is a Bondono and colleagues, I believe, a 2012 randomized controlled trial, and, as the name suggests, this was looking at apples that are going to be obviously a rich source of flavonoids, and then spinach, which are going to be a rich source of nitrates, as we've mentioned in our nitrate specific episode as well. So this was a crossover trial, both men and women in this, and looking at essentially four different treatments, the control group, the apple group, the spinach group, and then a combination of apple and spinach, and then, obviously, being a crossover nature people were crossed over into different groups, looking at a number of different measures, including nitric oxide as well as blood pressure, endothelial function, flow mediated dilation, and others that we've already mentioned. Some interesting results I think in this for you, what was the main thing that you took from the results of this trial?

ALAN FLANAGAN: For me, it was the fact that the impact on flow mediated dilation was higher for apple than it was, and it was kind of the next highest was spinach nitrates, and the combination of the two had less impact then either in isolation. But I thought the order of effect was interesting in terms of the apple, yeah, the apple rich, you know, flavonoid rich apple intervention having a greater magnitude of effect on FMD than the spinach nitrate. And then, interestingly again, with systolic blood pressure, although this change was, in terms of the difference, was negligible at like 3.3 lower systolic blood pressure with the apple 2.7 lower with the

spinach, but no significant change for both. So I thought this was interesting. There was no difference in oxidative stress really. But that might have just reflected the fact that these were 30 otherwise healthy young adults, and it may be that you might see more of an effect in a population with kind of higher baseline levels of oxidative stress.

DANNY LENNON: Right, yeah. And that's one of the cool things here that we are detecting these differences in this type of population, and the fact that it is more of a food based intervention is something to note as well. So yeah, I think for those reasons, that's an interesting one to mention in this respect. I don't know if there's too much else we need to go into on that particular trial.

ALAN FLANAGAN: Not that I can think of.

DANNY LENNON: Cool. Did you have any more that you wanted to bring up, or, are we good?

ALAN FLANAGAN: Not that I can think of. Yeah, I think we've covered a nice kind of spectrum of different types.

DANNY LENNON: Yeah, cool. And I think by now, hopefully, that's giving the kind of similar themes to what we were trying to get across through just taking a snapshot of a number of these different studies. And I think, hopefully, this fits in with much of what we had started last week in the polyphenol discussion. And it's, of course, an interesting area, and there's lots more of nuance we could get into, but in relation to these particular outcomes that we discussed this week and last week, hopefully, it gives people a good sense of what is currently out there. In terms of kind of general conclusions about this area of literature, what are some things that you might want to leave people with as kind of big key ideas or things to remember when they're thinking about polyphenols and their impact on health in at least the domains that we've looked at?

ALAN FLANAGAN: There's maybe a couple of levels that we can think of this. There's one that's a sensitivity to method in terms of nutrition science, nutrition research, obviously evolving the initial focus on the parent compounds, okay, no, it's probably the metabolites more focused on the metabolites. So this is an evolving area of research that reflects advancing methodology. I think there is a way then of thinking about the health effects in terms of the evidence as we currently stand, and that's linking up this kind of chain between the epidemiology, the evidence we have and interventions, and the biological plausibility that we could have from the mechanisms that have been elucidated. And they tend to me to join up. We, like I said, at the start, have long standing associations between flavonoids and cardiovascular outcomes. We certainly have, in the epidemiology of diet and dementia, they are possibly second only to long chain omega-3 fatty acids is one of the more consistent and strongest associations that will be observed. And interestingly, with the interventions, I think, for both cardiovascular disease and the cognitive health outcomes, what we've seen is effects across a spectrum of the lifespan and across a spectrum of health status of participants. With the cardiovascular outcomes, it may be that the effect is, with certain factors like blood pressure, risk factors like blood pressure, there's a greater magnitude of effect. But certainly, the fact that these kinds of outcomes are observed, not necessarily confined to either only people with existing risk factors, but also otherwise healthy individuals, does lends more support then to the kind of plausible biological mechanisms by which you might think the consistent intake of a polyphenol rich diet over time is something that, despite their lack of nutritional essentiality, which we discussed in the first podcast is a concept that people probably sometimes misinterpret. But this idea of being lifespan essential, I think, is something that does seem to hold up when these converging

lines of evidence tend to corroborate each other.

DANNY LENNON: Yeah, and hopefully, as people have seen, we've gone all the way from looking at some of the epidemiological associations that started very early as you mentioned, then into certain trials that looked at diet types, for example, that PREDIMED substudy, then to looking at certain trials where they looked at foods that are rich in these certain compounds, then all the way down to isolated components or specific compounds that are given as extracts or otherwise. And so, that's important to do, because I'm sure as people have probably already thought about, that foods, for example, that we are talking about as polyphenol rich have other aspects to them that could have similar benefits are going to be anti-inflammatory as one example. Right? If we take olive oil, that's not just a source of polyphenols, it's a source of monounsaturated fat, tocopheryl, things that are going to contribute towards an overall anti-inflammatory effect perhaps. And then even within those different types of foods, we need to kind of think about specific types of them, and their polyphenol content, so extra virgin olive oil will be different to a more refined type of olive oil because they have different levels of polyphenols as an example. So hopefully, again, seeing this across a number of different types of studies, and therefore, being able to hopefully give us a bit more confidence in one or the other, because we're seeing this convergence as you've outlined.

ALAN FLANAGAN: Yeah.

DANNY LENNON: Cool. Well, I think that kind of does this then for this topic. And hopefully, this adds to people's understanding and some of the things that we built on from last week, and obviously, this week, in the context of cardiovascular disease specifically. But yeah, I think that does this, if you're happy with that.