DANNY LENNON: Hello, and welcome to another episode of sigma nutrition radio. This is episode 411 of the podcasts. My name is Danny Lennon. And here with me is of course, Alan Flanagan. Alan, how are you today?

ALAN FLANAGAN: I'm good. I'm kind of taking into the final sprint. We were talking about in terms of writing up the thesis, but --

DANNY LENNON: And congratulations are in order for another published paper.

ALAN FLANAGAN: Yeah, so that was good news got that published. It was published today, actually. So, it's in nursing reports. So, that was actually from my MSc research, which was looking at the dietary intakes primarily in 20 NHS nurses, but we had data on their children, because the original research, which was a sociology PhD, was looking at the impact of shift work on a family unit, you know, within the family unit. And there was a range of kind of sleeping kind of measures like cortisol and stuff like that, that they took melatonin that was published previously. But they also did 14 days of diet diaries. So, we had the data for the children, dependent children and the partners and male

partners. And the initial nurse's analysis was interesting. But we kind of thought, we have this data and it was analyzed, you know, and it is interesting, because, you know, kind of brings in some of these more social and environmental factors. So, what we were basically asking was, well, was there an effect of the in-home diet on nights when mom was working shifts and was out of the home? What was, if any, the impact on diets in particular, we're particularly interested in the children, but we analyze the partners as well. And yeah, so what we found was that in pre-teen children, which in this study was 9 to 12 years old, that there was a greater proportion of their daily energy was consumed in the evening, during night shifts compared to non-night, so, but there was no difference necessarily in their total daily energy. So, what this was suggesting to us was that there was a kind of overall disruption to their patterns of energy intake on those days, whether that relates to say, for example, the fact that they're more dependent, so the teenage kids and the partners are able to kind of just like, continue eating as they otherwise would, or whether it's because, you know, mom is like, coming home at 8AM. And, obviously going to bed and so breakfast is left to dad, and, you know, he doesn't pull his weight, whatever was going on, we obviously can only speculate, but yeah, it was kind of, it was a nice for me, I kind of like I have obviously, as you know, we talk a lot about the various kinds of social and economic factors that influence diet. So, it was kind of nice to take an analysis in that kind of direction. So yeah, interesting, nice to get published.

DANNY LENNON: And if there's someone listening, who's maybe interested in doing their own kind of project in this area, what kind of questions off the back of that do you think would be interesting to look at that? You think that threw up in your mind?

ALAN FLANAGAN: Yeah, there was a couple of, you know, because obviously, a lot of the research even looks at shiftwork almost in this acute context, you

know, even from a dietary perspective, it's almost kind of, even if there are multiple nights worked that kind of tend to synthesize it into an, this was average intake. And the initial paper out of this that we published last year, actually showed this kind of dynamic redistribution of energy over other sequential nights. So, what I think will be interesting to look at from the perspective of the current paper would be some of these kind of in-home factors, what are the factors that go into, there was some evidence in the initial qualitative data from the thesis that the nurses would prepare for night shifts by, you know, preparing meals in advance, the run up period to night shift seemed quite kind of quite busy as they were preparing to, obviously be working overnight, have disruption to the normal kind of in-home schedule. And so, I think it would be interesting to maybe look at, you know, how that influences the kind of both their dynamics around not just the dietary intake, but what are the factors kind of influencing that in-home food preparation or, you know, how does it affect some of these kinds of aspects of, you know, who then becomes responsible because although the evidence suggests that there has been a shift in households in terms of primary responsibility for example, food budgeting and shopping, as in there is more male participation in that.

Overall, the evidence still shows that that women in the home still are more likely to bear primary responsibility for things like food budget and stuff like that. So, I think it would be interesting to probably dig in kind of more from a qualitative perspective even looking at some of these factors, how do the dynamics of accounting for, you know, household nutrition change over periods of nightshift work? And what are the implications then for particularly children? So yeah, looking at like, you know, does dad start doing the shopping? If so, like, does the composition of foods change? Is there an impact on other meals like breakfast and lunch? I would have liked to have dug into that

a bit more. There was a suggestion in our data, which was unpublished a breakfast in the pre-teenage children was later as an in terms of timing, and that they had slightly kind of less energy at breakfast. So, you know, there was some suggestion in there that perhaps breakfast during the periods of nightshift work was effective. So, what's affecting it? And what were those changes, we didn't have enough data to kind of dig into that ultimately in any more detail? So, we were just looking at the redistribution of energy. So yeah, I think there's a lot there. You know, I did a lot of searching to kind of try and find was there any previous research that had looked at these kinds of questions like, what's the impact of an exposure or an occupation, like shift work on the rest of the family from a dietary perspective and nutrition perspective, and there is literally nothing. So, I think there's a lot of scope and low hanging fruit to be picked on a question like that.

DANNY LENNON: Yeah, especially when you say there's little out there currently, because presuming that there's maybe also some interesting differences based on what that family unit looks like. And like the ages of the kids, the number of kids, whether we're talking about single parent versus two parent households, all these different factors, presumably you might see some interesting things pop up?

ALAN FLANAGAN: Our study specifically recruited kind of two parent households, I think it will be interesting to look at what would there be more of an impact in single parent households. You know, our analysis didn't suggest that like, there was much of a difference in terms of say, within a family, because we did have pairs of siblings, but it was quite imbalanced. So, we couldn't really dig into that as much. But preliminary kind of analysis of that didn't suggest that there was much difference between say, boys and girls, or between say, kids within a family. But again, that could just be a reflection of our relatively small sample size. So, those questions

I would say would remain open. And so it will be interesting to kind of look at that. And certainly that the suggestion that we've offered from this kind of analysis is, you know, perhaps it's more dependent children. So like, you know, children, pre-teen to children that are perhaps more likely to be vulnerable to kind of disruptions within the household routine.

DANNY LENNON: Sure. Awesome. So, I will link up to that in the show note. So, everyone can go and take a read, which I encourage you to go and do. With today's topic, we're going to be talking about bone health, generally, and particularly the role of nutrition. Of course, bone health has many factors. And we'll maybe touch on a few that can influence that. But we'll be zeroing in on some specific nutrients and the implications for different levels of those. And we'll walk through some interventions as well. Maybe to set the stage a bit of a quick introduction, around some basics of bone metabolism, some definitions, how nutrition factors into this whole bone health issue from like an overview level, and then we'll obviously dig into the details later on. Probably what were from a long term perspective kind of mentioned a number of times is risk of osteoporosis. So, this being defined as a systemic skeletal disease characterized by low bone mass and deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture. And those kind of fragility fractures are really that the hallmark of osteoporosis essentially, they're particularly common in the spine, in the hip and the distal forearm. That's just something to note, if you are looking at some of this research, that's why you might see certain sites commonly come up as ones that are being examined, although those fractures can occur elsewhere too.

Beyond osteoporosis, we also have osteopenia, where bones have lost mass and are weaker, but not to the point where one has osteoporosis. So, maybe we could think of osteopenia being to osteoporosis what pre-

diabetes is to diabetes in some sort of fashion. So, with this low bone mineral mass being the main factor underlying osteoporotic fracture, we know that bone mass later in life is going to be dependent on two things. And this is important to keep in mind when we're thinking of different types of trials. One will be the peak bone mass that someone achieved early in life. And that peak bone mass typically is by the age of 30, although 90% of that is by the age of 20 typically and then so once that peak bone mass is established, that will be one factor that will dictate maybe someone's bone mass later in life. But the second part of that, which is hopefully maybe one of the modifiable things later in life will be that rate of age related bone mass. And so we can look at interventions that look at both. So, during childhood and adolescence, what contributes to someone achieving maybe the highest peak bone mass that they can. And then some of the interventions that are later in life, how do we kind of slow down losses of bone. With this, there's obviously a huge burden of disease. Some of the statistics that I was able to find on this show that osteoporotic fractures in the US contribute up to 1.5 million people suffering these fractures each year, you see a similar burden in the UK as well.

I think there was some of the epidemiological studies, hypothesizing that one in two women and one in five men aged 50 years will suffer an osteoporotic fracture at some point in their life time. And this translates then also to some of the economics, you see an economic burden of osteoporosis for that fracture in the US being estimated around 18 billion per annum. And that's 4 billion pounds sterling in the UK per annum for an estimate there. So, the kind of final thing I'll mention before we start digging into some of this is that while we will have a kind of focus here on diet and nutrients, there are of course many factors that will influence bone mass. Some of these cannot be modified. So, these are things like sex, for example, where you see higher prevalence of osteopenia

and osteoporosis in women age, as we all mentioned a number of times, genetics plays a role here. And typically you see people with a family history, that puts them at a higher risk. And then we see differences in ethnicity as well. So, osteopenia, for example, being more common in Caucasian or Asian populations. So, if our focus, which is to modifiable risk factors, then there's kind of maybe three broad categories we can think of probably two is where we're going to focus on. First, if we think of someone's hormonal status, we can look at both sex and calciotropic hormone status. So, the sex hormones and as we'll discuss in relation to menopause later on, we can look at estrogen and its role in bone. And then calciotropic just simply means hormones that are involved in calcium homeostasis. And so the ones that will maybe mention at some point would be 1,25-dihydroxyvitamin D, which is an active form of vitamin D and then parathyroid hormone. So, just a note that those may be referenced later on. Various lifestyle factors can have a role here, physical activity, smoking, alcohol, and then of course diet, which includes food fortification, functional foods, supplementation, as well as overall diet. And that's obviously going to be our focus here today. So, that kind of sets the stage of why we are talking about this issue. And the kind of importance of this from not an individual level, but a public health level. Anything you would add in there, or you want to emphasize is particularly important Alan from the introduction?

ALAN FLANAGAN: not particularly, I think, just the concept of the importance of nutrition, like you said, there are so many other factors that are important for bone health, you know, mechanical loading and all of this, but for the adult period that we're tending to focus on, it's this reality that there's constant bone remodeling over this period. And so, although there is this inevitable kind of age related decline in bone mass, if I remember starts from around 34, 35 And that becomes accelerated with certain variables like

menopause, through this period, you have this constant during adulthood, you know, remodeling of bone and new bone replacing kind of older bone and so you have this constant, essentially requirement for these base levels of essential nutrients that are important for sustaining and maintaining the skeletal integrity.

DANNY LENNON: Yeah. And people may see kind of this, kind of common fun fact of like, we basically replace our skeleton every like nine or 10 years something like this, because of that bone remodeling this constant bone turnover that, every decade your skeleton is essentially completely different from what it was, you've changed every single bone cell by that point from decade previous, which is kind of wild to think of. So, the bone remodeling is actually a good point of departure to jump into the kind of roll of why we're talking about the nutrients that we are going to talk about. And so with this process of bone remodeling, we have this kind of breakdown of bone. And then this kind of building back off of bone to have this constant bone turnover, there's a couple of different cells that are worth pointing out here, because they may crop up later on. So, we have in bone remodeling, we have that removal of that kind of old bone or that kind of resorption, or breakdown of bone by these cells called osteoclasts. And then we have the formation of new bone primarily being driven by osteoblasts. And as a simplified way to think of it but for our purposes, we can just think of it like that, that these osteoclasts are driving breakdown osteoblasts being related to the formation of new bone. And so we're kind of maintaining this bone homeostasis by balancing this bone resorption by osteoclasts and bone formation by osteoblasts. And kind of a way I remember when I was in college of trying to remember those is like the osteoblasts, the B being for building and then osteoclast, you can think of like the C is like catabolic or catalyzing is one way to maybe memorize those.

Now these will come up when we talk about maybe mechanistically why certain nutrients. And in particular, probably the main ones we'll look at will be calcium and vitamin D, and also a bit around vitamin K as well. These all have roles here in influencing some of the process of bone turnover and having roles may be influencing these cells as well. So, just that might be some stage to set in mind. So, maybe that start with calcium and or combination of calcium and vitamin D, because some of the trials will often do a combination of those. But if we put our kind of focus on calcium to start here, how would you typically frame this of why this is a nutrient that's looked at, I suppose probably this is the most obvious to most people I think, if you asked most people in the general population, what nutrient maybe is for strong bones, people have been drilled into them about calcium. But what is actually this kind of role that we can have? Why is calcium of interest here?

ALAN FLANAGAN: Primarily because I mean, over 90% of the calcium that we have is deposited within the skeleton, and well and also teeth as well. But the skeleton is the primary, we don't tend to have much by way of circulating calcium. This is relevant when we consider studies like Mendelian randomization. But in general, you know, we want calcium to be in the skeleton. And so as a primary structural component of that, I mean, ultimately, bone is primarily composed of collagen, type I collagen, and obviously the bone cells that you've mentioned. But then the primary mineral content of bone is calcium and phosphorus, that add kind of mineral density and strength to the actual bone itself. So, it is of primary importance in just at the level of the requirements for calcium structurally within the bone. And calcium has been obviously a source of focus in terms of not just public health recommendations, like you said, if we lined up 10 People who know nothing else about nutrition against a wall, you know, they'd probably be able to know, oh,

yeah, calcium, and they'd probably be able to make a kind of mental link to, you know, perhaps I don't know, ads for milk or whatever. But it's almost a classic example of why studying nutrients from a research methodology perspective is quite challenging for nutrition science. Calcium has a number of factors that influence its levels in the body, vitamin D up regulates calcium absorption at the level of the gut, we know that's one of the primary functions of active vitamin D itself, dietary protein kind of enhance calcium bioavailability by at the level of the stomach increasing acid secretion, which is required then to kind of cleave off and make calcium kind of available for absorption. These are factors that perhaps modify the effect of calcium both through diet and potentially supplementation, it can be difficult to isolate those effects.

And then there's also similar even to dietary protein recommendations, some of the general recommendations that we have for what are considered calcium intakes that will be beneficial come from calcium turnover studies. There's more recent evidence to suggest that perhaps they're not entirely capturing the full picture of calcium requirements and the actual kind of minimum requirements may be lower than some of these, you know, suggest kind of a range of 1000 milligrams to 1200 milligrams a day. And indeed, that's where most of the supplemental intervention have targeted a dose, but there's, you know, evidence to suggest that perhaps actually somewhere around 700 milligrams of dietary intake would be sufficient. So, calcium itself is in and then finally, as a last consideration that there is this difference, potential difference between calcium consumed through the diet in the context of say, adequate vitamin D levels and a higher dietary protein level. And also food sources providing that calcium versus the effective and isolated supplemental nutrients. And of course, that then relates to someone's baseline levels of intake.

So all of these kinds of methodological challenges that we've talked about before all reared their head, when we're looking at calcium and kind of and bone health. And it does make for confusing, kind of understandably so evidence base in relation to some evidence of benefit, some interventions not showing a benefit, some showing a benefit in the context of effect moderation, or perhaps low levels of baseline intake and stuff like that. So, I think there's a number of those factors that kind of rear their head and the calcium and or calcium vitamin D, and or calcium plus protein research.

DANNY LENNON: At least for me, I'd be interested to hear your thoughts, one of the things that pretty much jumps out, as you start going through some of this, this data is that you almost have to take individual questions around calcium, and its impact on bone, because there are so many different ways to look at it. And you've kind of touched on a few of those of, we could have interventions of like overall dietary intake and say early in life and how that contributes to achieving peak bone mass, that would be a very different question than to looking at a supplemental intervention later in life to try and rescue some lost bone as one example. And then we have some of those methodological issues that you highlight of, okay, are we actually measuring measured intake from someone's food? Or are we looking at something like serum calcium levels and trying to draw a kind of conclusions from there? And does that throw up differences in how we view some of this different data? And so there's just so many ways to kind of get at this question that looking at just one is probably not going to be that productive. And then there's obviously specifics when we dive into some of these studies. And so from that perspective, what might be the best place for us to start in terms of looking at this question of, if we do want to mention any studies in particular, we don't have to, we could just give an overview, but

like, what is the best way to go about this, do you think?

ALAN FLANAGAN: I think because of the overlap between calcium and vitamin D, and the fact that more often than not they're used concomitantly. That's probably the point of departure, I think would be if we're just going to think about calcium specifically for a second, assessing the role of calcium in isolation is difficult, because you tend to have concurrent use of supplemental calcium alongside varying doses of vitamin D, and RCTs. And again, these varying doses of vitamin D also relate to an individual's background, baseline 25-hydroxy vitamin D status, and you know, what levels are achieved from the intervention. But in trying to isolate the effects of calcium alone, there was a nice 2007 analysis.

So, it is kind of older at this point, but it puts this in context analysis by Boonen and colleagues where they were separating RCTs that used only vitamin D, or RCTs that used vitamin D plus calcium. And the ultimate conclusion of that analysis was that reduction in fracture risk was primarily only observed when calcium supplementation was concurrent alongside vitamin D supplementation. And that was at varying levels of vitamin D from kind of 400 to 800 international units of D3. So, it may be that in isolation, there is potentially little effect. And there's some recent prospective data to support that. There's a really interesting study last year Bristow and colleagues in JCEM Journal of Clinical Endocrinology and Metabolism that basically looked at dietary levels of calcium intake, stratified into different quintiles of intake and looked at BMD over six years. And so the levels of intake in this study, you know, varied from kind of less than 596, nearly 600 milligrams a day to over 1100 milligrams. And there was really no difference across any of these in terms of BMD over this six-year period. And the mean intake in this whole group was about 886 milligrams a day. So, what we could conclude

perhaps is that one, the levels of intake that have previously been suggested to be needed through diet may be lower than previously thought, based on calcium kind of turnover studies. The second could be that in that range of dietary intake, it's sufficient, even though that stratifying the quintiles, you know, didn't show much of a difference between these groups. But with this average intake in kind of women over 70, that there was, you know, little effect of calcium in isolation at that level of intake.

Now, you could say, then, that kind of negates any benefit to calcium supplementation. But in terms of getting granular at some of this evidence, you do tend to see an effect of calcium plus vitamin D supplementation concurrently in particularly care home elderly settings. So, in the over 70 age group and people who are confined to care homes, there are other risk factors that come into care homes, like just lack of mechanical loading and stuff like that just sitting in a chair all day, this kind of thing. But there does seem to be in terms of the wage of evidence that looks specifically at that kind of end of the lifespan evidence that, yes, a supplemental dose of 1000 to 1200 milligrams of calcium and about 800 IU of vitamin D3 a day is protective against fracture risk. And that higher dose of calcium and the potential benefit to that adjuvant to vitamin D or concomitant with it may simply reflect the fact that in that when you measure calcium intake in that age group in a care home setting, it tends to be very, very low. It's primarily derived from, you know, the milk in the in the cups of tea throughout the day and there is and so you might get this benefit in that age group. But if we're trying to isolate the independent effects of calcium, it does appear that overall there's a kind of an effect modification of these other factors. And potentially now, in terms of kind of more recent evidence, levels of intake and dietary in a range of say a minimum of 700 to 800 milligrams appear to be sufficient in a younger

demographic, although that Bristow and colleagues study was in women with an average age of 70 at baseline, but that's high enough, potentially high enough calcium intake to kind of protect against the BMD loss.

DANNY LENNON: Yeah, super interesting. I suppose there's a couple of elements that we can dive into, I think, if we're thinking of this combination of calcium along with the food matrix within it's consumed, and then also some of these other nutrients and you've mentioned vitamin D, but also previously had mentioned protein, one of the papers that we've mentioned beforehand, before recording here was a paper that actually just been published yesterday at the time of recording really cool paper out of Australia that was in residential care facility. So again, that kind of demographic that you just discussed of having maybe some of the biggest benefit. So, this was a two year randomized control trial across 60 residential care facilities in Australia, you've around 7,000 residents with a mean age of 86. And they were all had adequate levels of vitamin D and that was maintained through supplementation importantly.

And so the intervention here was to in the actual intervention group, they had an increase in dairy food consumption. So, going from two servings per day to three and a half servings per day from milk, cheese and yogurt. So, what that ended up being was about 250 milliliters of milk, 20 grams of cheese, or 100 grams of yogurt. So, that was an increase of 562 milligrams per day of calcium, but also an additional 12 grams a day of protein, and then you have totals for the day, then we're being around 1100 milligrams of calcium and around 70 grams of protein per day, which equated to about 1.1 grams of protein per kilogram of body weight. Compare that to the control group where you had their usual menus that they said consuming, which was about 700 milligrams of calcium a day as opposed to 1100 milligrams plus, and then 58 grams a day of protein, which is about 0.9 grams per kilo of body weight. And

the primary outcome they looked at here was fragility fracture had secondary outcomes were time to fall and changes in bone morphology. Across that study, we see 324 fractures occur, over 4,000 falls and close to 2,000 deaths in the time of this RCT. And of those results, we see pretty big risk reductions, 33% risk reduction for all fracture, 46% for hip fracture, and 11% for falls generally. So, this is where we're starting to see pretty significant implications here of this food base change of increasing that dairy food intake, where we're getting not only increasing calcium, but also of protein in this group that we've kind of acknowledged is in the kind of this kind of care home setting. And so there's a number of factors going on there that may relate to not only the two nutrients and combination, but also the fact that we have this specific population as well. And then also, the additional of this is not a kind of a supplement trial. This was from food. And so this is often one of those questions of like, particularly with dairy and things like cheeses and the food matrix it's in, there seems to be something sometimes it kind of unique as opposed to just isolate the calcium, as you had mentioned.

ALAN FLANAGAN: Yeah, absolutely. And I did this, reading this, I just had this paper jump into my mind from the 2003 paper from Best Olson Hughes, and it was in the American Journal of Clinical Nutrition. And they were looking at secondary analysis of the women's health initiative intervention. And essentially, what they were looking at was this specific question of, we know that protein appears to have a kind of a modifying effect on calcium bioavailability and absorption. Is there a potential effect modification on actual outcomes like BMD? And so their analysis was based on a trial where the supplemental intervention was 1000 milligrams of calcium and 400 IU of D3 a day, but they stratified the women in the study by levels of dietary protein intake and found that the greatest preservation effect on BMD was in the supplemental group with the highest

dietary protein intake. And so that was obviously focusing on a supplemental intervention. But it jogs my memory on that study, because of this kind of interactive effect between potentially dietary protein and calcium and then bone health outcomes. And prospectively, we've seen this more recently in the UK with a study that was looking at vegan diets, for example. And, you know, finding that that actually dietary protein was one of the strong kind of moderating factors in the risk for fractures, significant increase in risk, I think over 50% in the vegan population in that study.

So, it's possible that, again, you know, that the effect of calcium in isolation, if there is an effect, whatever the size of that effect is and that obviously differs from study to study. But certainly the effect modification by dietary protein as a bit of a lineage now in kind of the study that I was mentioning, the Dawson Hughes study was 2003. And so we have this study coming out. Now, that's obviously a food based intervention, where really, it's this kind of combination effect of enhance of increased dietary protein plus added calcium in the vulnerable population group at which we've primarily seen most of the benefits of supplementation previously.

DANNY LENNON: Do you have any thoughts around some of the data that looks at serum calcium levels and in particular, I know, ones that would be interested to hear your thoughts on is a couple of the recent Mendelian randomization trials where they looked at serum calcium levels, and I think total body bone mineral density. And there again, you'll kind of see this thing of like, those serum calcium levels, differences in those so like people who genetically had higher serum calcium levels compared to lower didn't seem to have like better outcomes in terms of BMD in the general population. I think except maybe when you looked at people over 60, then you started to see some differences. But I think there was actually a slight reduction. Now again, how strong that was, it doesn't matter.

But essentially, some of this suggesting, well, based on someone's genetic kind of serum calcium levels going around, we actually don't see any real difference. I'm wondering how you think that type of data kind of fits into the bigger picture of what we're actually talking about with like nutrition trials?

ALAN FLANAGAN: Yeah, I've been thinking, still have yet to come to kind of hard and fast conclusions. This is a kind of an ongoing thought process in relation to the use of Mendelian randomization studies for nutrient exposures. One thing I'm certain off from understanding the assumptions that go into whether an instrument variable is valid and therefore whether a causal inference can be made from an MR study? Is that there are really gratuitous causal claims being made almost, you know, with the similar kind of reification of meta-analysis that, oh, it's a meta-analysis, its findings therefore, you know, are on top of the pyramid. I think there's a similar kind of oversimplification in the published papers themselves. And rather kind of gratuitous use of causal language. MR is often kind of compared to RCTs, that's probably not an accurate position of them on the pay, if you were to insert Mendelian randomization on the hierarchy of evidence, they would be kind of above our observational, you know, prospective cohorts case control studies, but they would still be below systematic review, meta-analysis and RCTs.

Now, if all of those assumptions hold true for an instrument variable, well, then they get elevated, you know, to potentially being on a par. If they don't hold true, then we're just talking about associations, right? So, this is a really important distinction. So, people say, MR causal, there's like, no, if there are certain factors that we can't satisfy, then an MR is an association study. Now, why is this relevant for in this instance looking at serum calcium levels? Well, even if and this applies to one of the issues with MR is this idea of playtropi (ph), right, that the fact that something could

have act through multiple pathways to be a valid instrument variable, an instrument variable and MR meaning, you know, the thing you're looking at the gene exposure, for example, well, then you want that to be an estimate of the risk factor of the exposure. So, say genetically higher or lower, whatever it is, on your outcome, disease, or whatever, and you want that and need us to be unbiased for many unobserved confounding factors. But you still need your instrument variable to be valid, you need a couple of conditions to be met, relevance exchangeability and what they call exclusion restriction.

Now, without delving this, I think this is probably something we can cover kind of in a specific episode in future. But relevance is really important here, because nutrients are what we would call polyvalent, right? Nutrients act through multiple tissues and pathways like that. Let's just take another example like Omega-3 Fatty Acids, right, there's a role for them in the nervous system and neurological health, there's a role for them in cardiovascular health as a role, you know, in terms of the liver and hepatic, you know, fatty acid metabolism, all this kind of stuff. So, relevance is really important here, because whatever variant you're looking for, has to be kind of directly related to the risk factor and the outcome. And when it comes to calcium, serum calcium is generally maintained within really tight homeostatic ranges, right? There isn't this wide variance. It's not like exogenous levels of a nutrient that just, you know, kind of directly reflect intake, like we would with some nutrients that are not endogenously synthesized in the body. Calcium, like we said at the start is primarily stored. And so with MR, although there can be these processes that the researchers go through to satisfy themselves that as an instrument variable, it is valid in terms of being the risk factor associated potentially with an outcome unaffected by potential confounders. The difficulty then is, are we sure that serum

calcium itself is the marker that is important? Or is it skeletal calcium? And so that's where we get into difficulties. There's a number of examples for this for nutrition. I mean, it's been a point of controversy in relation to vitamin D research. So insofar as what we know about calcium metabolism in terms of the tight links, these analyses have been looking at serum levels of calcium and relating it to BMD and ultimately saying that, you know, there's either a potential negative impact on bone metabolism or no effect at all. Ultimately, when I'm looking at these, I'm just not convinced that serum calcium is necessarily a relevant marker for the effect of calcium in bone and consequently on long term bone health related outcomes. So, I'm very cautious in looking at and I would encourage people to be cautious and skeptical with looking at a lot of MR studies coming out with nutrient kind of based analyses, or the proxies that they're looking for, and certainly be very skeptical when causal claims are being made, which may or may not reflect, because the ultimate conclusion with these causal claims is that there will there's no causal link between calcium intake, that this is the deductive step that's made. That's not really what's being shown.

DANNY LENNON: Right. And it may be that I'm just too dumb to get or I'm completely missing something. But for me, when I started seeing, like serum calcium stuff, it's, as you say, many things could dictate that. And for example, as we'll probably come on to in a moment, if we're thinking about when there's an increase in, say, the secretion of parathyroid hormone, you tend to see increases in calcium blood levels. But what's happening there is it you're getting this increased mobilization of calcium from bone, so that parathyroid hormone is contributing to this bone mobilization is breakdown of bone, which is ending up in serum and you're seeing this increase level. And so even from that, I was like, I don't know, like, sure, that's isolated situations, but you, then theoretically, if someone has hyperparathyroidism, you will see

increased serum calcium levels. And so yeah, that's not necessarily that this person is consuming enough calcium for their needs. It's reflective of something else.

ALAN FLANAGAN: Yeah, exactly. And even there's issues with MR making assumptions that then relate to intake, you know, I saw an MR, Mendelian randomization study that was like, genetically predicted coffee consumption. I was like, what is that coffee consumption? Do I have a genetic predisposition to consumers the amount of coffee I do? Like I think we need to be just a bit more, it's obviously an exploding area. And because of the kind of open availability of these genetic data bases that you can, you know, genome wide association studies, and you can find your, your gene you want to look at, but we have to remember that these are often proxies. Again, this is kind of these are kind of auditor comments, so to speak. But, you know, sticking to the calcium example, you know, to make the kind of inferences that some of these studies are trying to conclude, you need to be certain that it's actually serum calcium. That is a relevant biomarker of calcium, as it relates to these other factors. And when we consider how tightly regulated in within a homeostatic range serum calcium is. The body doesn't like calcium floating about the place. That's why it's primarily stored in our skeletal, skeleton and teeth. Calcification is obviously a process that is kind of implicated in some kind of adverse processes, particularly for cardiovascular health. Again, I'm kind of very much withholding any sort of competent conclusion that MR of serum calcium is necessarily reflecting the causal relationship between skeletal dietary calcium or any of these kinds of BMD or fracture related outcomes.

DANNY LENNON: Cool. So, with that, and for the sake of time, let's jump into some of the vitamin D literature specifically. And there's, obviously at this point, a considerable amount of evidence in relation to vitamin D deficiency, being an important contributor to things like osteoporosis, one of

those ways that we'll probably discuss is around the absorption of calcium. And that's why there's kind of this, looking at these two things in combination. But there's also maybe some other vitamin D specific issues that we can discuss too. With vitamin D deficiency and we discussed some of this in our vitamin D episodes, so people can go and refer to that is that we can have situations where there's an inadequate mineralization or even D mineralization of bone. And in the episode we talked about in children, we see this severe vitamin D deficiency show up as rickets. In adults, we see something called osteomalacia form, which is essentially kind of translates as soft bones, I think, in a literal setting, malacia being the kind of Greek for soft, and of course, osteo relating to bone. So, we have this kind of weakened bones and more susceptibility for them to break. So, we know those are kind of consequences of a true vitamin D deficiency. And then, as we had just maybe alluded to a few months ago, with low vitamin D status, we tend to see that lead on to secondary hyperparathyroidism. And so that the parathyroid glands here, which are located in the neck, near the thyroid gland, produce too much of parathyroid hormone. And then that increase then causes an increase level of calcium in the blood, which is essentially this enhanced mobilization of calcium from skeletal bone, which we just mentioned a moment ago. So, with that, and those kind of clear associations with vitamin D and things like rickets, osteomalacia, osteoporosis, we can maybe start looking at some of the actual trials that have used vitamin D, because I think these might be useful to illuminate a couple of key themes that kind of keep emerging in this area of literature. I think we may have hinted at some of them in relation to vitamin D's impact on other various disease states in our vitamin D episode. But we kind of kind of see some of these themes continue in relation to bone health here as well. So, there's maybe two or three of these that we can maybe dive into. Is

there any that you want to pick first out of the ones that we've highlighted?

ALAN FLANAGAN: Yeah. Well, I think that the contrast between New Age and that's an acronym for people listening a New Age cups. And the vital trial is an interesting juxtaposition between these two studies, the doses used, the outcomes that highlights these themes that we're thinking of, and then these seem specifically relate to something we've talked about a lot before, which is there's no zero exposure in human nutrition. There's no treatment and true placebo, right, because that assumption that you would have for biomedical RCTs assumes the treatment is a drug that is not otherwise in the participant system. And the placebo then is a true zero exposure, there's no bit of the drug, they're not eating the drug in their diet or getting it from being out in sunshine in this case. So, the challenge is that nutrients exist on a bell curve from insufficiency to adequate to potentially access. For the most part, we tend to be concerned with insufficiency to adequacy, particularly as it relates to vitamin D. And the background status of your participants is going to be an important determinant of potential determinant of the effects of your outcome. A New Age was published about three or four years ago now. And it was supplementing basically 400 international units of vitamin D. In elderly adults, it was a multicenter study across Europe. And there were on average, about 70 years of age at baseline. And there was about 1,000 people in the trial that finished it and it was only a one-year trial. And ultimately, the conclusion was that this kind of they were recommended to follow a Mediterranean style diet, but the supplemental vitamin D additional had no effect on bone mineral density.

But then you look at the level of the supplement 400 IU, now that's in line with current guidelines. But there's a fairly overwhelming consensus that's probably like kind of throwing a spotlight at a tank, as far as

making a dent in vitamin D levels in the body goes. The baseline levels of vitamin D in the study were 24 nanograms per milliliter in terms of millimole per liter, that's about 60, right. And generally in the UK, 50 or over is considered give or take around between 50 and 75. Give or take where you would want to be according to kind of UK and certainly European guidelines. That's the kind of range of intake considered adequate. And this effect of 400 IU of vitamin D3 supplementation changed over one year, 25-hydroxy vitamin D. So, there's vitamin D status by 4 nanograms per milliliter like that that's all it changed. It went from 24 nanograms to 29, right.

So, the conclusions that you get from studies like this have to be taken in this context. Are we saying that this is a no trial in relation to vitamin D, ie, there was no effect of vitamin D supplementation? I don't personally think that's a conclusion we can ever just make simpliciter in relation to nutrition RCTs. This is something we've talked about a lot. Really the conclusion here is that in participants with potentially adequate vitamin D status, giving them so little vitamin D that their vitamin D barely changes over the course of one year did not change bone mineral density outcomes, rather than saying that vitamin D itself had no effect because it can't be taken independent of those factors. Their vitamin D status basically didn't change to any meaningful degree.

And then you have vital, which kind of comes to the other end of the spectrum, right. Vital was 2000 IU of D3 a day with baseline levels of 25-hydroxy D of 75 nanomoles per liter. Now in the US and North America, this is a debate within the kind of vitamin D research in the US and North America. They say 50 to 75 is still insufficient. 75 is where you get this maximum kind of suppression of parathyroid hormone. That's actually what we want. Therefore, over 75 is preferable. There's an ongoing debate over that within the vitamin D literature. But we've got people with already give or take around

about 75 nanomoles per liter, or 30 nanograms per milliliter range. And there was an increase from the 30. If we're talking nanograms to 41, or in millimoles, they went from 75 to about 104 millimoles per liter. So, they've gone from high to higher. And so potentially the lack of effective vitamin D overall and vital in relation to falls. And these kinds of related bone outcomes could represent the opposite end of the spectrum. If New Age simply didn't really have any meaningful change in vitamin D in participants already sufficient, vital to people with already way higher levels of vitamin D that you would generally get in the average UK population, for example, bumped it up even higher than high and still found no effect. And it's illustrative of this point, that once within a range of adequacy, more of any given nutrients does not necessarily equal better. We're not talking about drugs that you're just titrating a dose with. So, if you already have sufficient levels, the fact that you have a null outcome may not represent the effect of that intervention. And then these are examples of why it's really important to have levels of intake where your participants are genuinely deficient at baseline. And you bring them up to these sufficiency ranges. Otherwise, how are you expected to see any effect?

DANNY LENNON: Right. Yeah. And there's the debate around what threshold we should see as sufficient is kind of important here. Because I know within that vital trial, part of some of the kind of sub analysis, if I have correct was, they did compare those that had baseline starting of less than 50 and over 50. Now, if we're going with like North American kind of guidelines of anything below 75 is insufficient. So, anything below 50 is like really bad, then you would expect to see a big difference there. If we're going with anything above 50 is actually sufficient, then even if someone is a bit below 50, they're probably like, not terrible in terms of vitamin D status. And so you might not really see much of a difference in comparing that. And then I guess the other thing that kind

of jumped out to me there is that we were looking at people 50 and over in this trial, around five years of kind of follow up. And with the main outcome being false, there's obviously then other things that might kind of go into that. And so we could actually be seeing differences maybe in bone, but just it doesn't end up being leading to differences in falls being measured, particularly when we look at kind of the age group here, it wasn't one of these over 75 or over 80 kind of cohorts, this is 50 plus. And then we're looking at the main outcome being differences in fall, right. So, I think those were things that to me, I would just at least make a note of.

ALAN FLANAGAN: Yeah. As a kind of an anecdote. This was pre COVID when, you know, kind of university was still functioning as normal and there was one of the nutrition departments kind of seminar. And Professor Susan Lanham-New, who's the head of the nutrition department at Surrey, but is prolific vitamin D researcher was talking about this kind of issue in relation to a grant application that Surrey who do a lot of vitamin D research. If I remember correctly, it was going to look at both Caucasian and South Asian populations, vitamin D supplementation and they specifically live to this issue, if I remember her comments correctly, wanted to recruit as is because in the population, there's such a prevalence of insufficient vitamin D status that they didn't see it as an ethical barrier. Well, we can recruit people who are naturally deficient and then we can intervene. And the ethics committee said, we'll know if they're deficient, you have to you have to bring them to sufficiency before the intervention. So, people are out there going, well, why does this happen? Like it's not that researchers are not live to this issue. It's often that ethics committees simply won't allow you in an intervention to have people or certainty to deliberately put people at risk. Now, this study wasn't looking to deliberately put people at rest. I think that's the real frustration is there such a prevalence of insufficient vitamin D

intake, we can definitely get people in the population and screen them and have people who are insufficient or deficient at baseline then intervene with a supplement. And yeah, so, you know if like, I hope my memory is doing me service in terms of those comments, but that's certainly how I remember it being articulated as really the barrier, what was the ethics committee in terms of insisting that people have to have like sufficiently baseline levels of 25-hydroxy vitamin D? Well, then how are you going to see an effect? And this pervades nutrition, intervention research.

DANNY LENNON: Yeah. And if people listening are actually looking for more detail on that specific issue. They go to Episode 396. I had Dr. Leigh Frame on and we kind of talked about some of this within nutrient trials and ethics, and the use of placebo groups and exactly that example that you give of how do we get around a situation where, if we know people are starting with adequate baseline levels, and particularly if the supplemental dose isn't really going to move the needle much on that, then why would we expect to see differences? And then so how do we tackle that from ethics standpoint of actually getting people who are deficient? And then we talked through some potential solutions, but again, you're still at the mercy of an ethics board to say, yeah, we think this solution is good. So, for example, the use of rescue therapy is something that isn't super common within nutrition, but could be a potential example, where you take that group of people and you are allowed to look at that period of time where there's insufficiency with a guarantee that on conclusion of the trial, they're all brought up to an adequate level, just as one potential solution, but whether a specific ethics board goes for that or not as another question, I guess, right?

ALAN FLANAGAN: Yeah, absolutely. So, it would be really helpful, particularly because we know from single nutrient insufficiency that, you know, adverse, or potentially adverse effects of insufficient

nutrient intake tend to be quite rapid onset. But there's also rapid recovery. They're short latency conditions. Now, that's not saying you're going to give people scurvy, and then be like, ah, here's some orange juice. But part of the problem is to an unknown, critical eye, or even just the passing observers, because everyone's interested in nutrition, that this is the source of so many talking heads being like, oh, we can't trust nutrition science. You know, it's the source of so many of the biomedical purists being like, oh, nutrition is unreliable. Look, these RCTs found no findings relative to epidemiology, but they don't have the subject specific matter knowledge to even understand why the RCT may be the one that's not.

DANNY LENNON: That's not the problem.

ALAN FLANAGAN: And the RCT is the one with the problem here. That's not, you know. And so these kind of gross straw man assumptions are made purely at the level of methodological prejudice that the RCT must be right, because it's an RCT, and factor in none of these underlying issues. And, yeah, I basically need to get that paper wrapped up and submitted. This is really important for vitamin D, because when we have looked at some of the, particularly the residential care setting groups, where again, you can naturally find people who are deficient, some of the older trials, you know, that kind of 90s and early 2000s, did find a benefit, when you take people with these low levels, you know, 15 nanograms per milliliter, and you bump that up, and then you see, you know, a benefit in some of these interventions. So, it's so crucial. And I guess our take home point would be to kind of remind listeners, as I'm sure they're familiar with, we beat on this drum a lot. But a no finding does not necessarily mean no effect of whatever the supplemental intervention was, in this case, no effect of vitamin D, it may simply be that there was no effect. Because the characteristics of the participant, they already had adequate levels of vitamin D or they had adequate levels of the new or the supplement

didn't change their already adequate levels, or even already deficient levels, you know, if it wasn't brought into a range of benefits, so it's all of these factors needs or mandate that we come to more nuanced overall conclusions rather than this study had no effect, because that's not really how we should characterize the conclusions or the findings from any of these interventions.

DANNY LENNON: Yeah, it's interesting to see how that is quite common, unfortunately, amongst this area of literature. And one of the other studies we had mentioned was a 2019 randomized control trial, this time looking at actual bone mineral density as opposed to falls. But this was a three-year trial in Canada. But the same kind of issue here is arising when you look at the baseline levels of 25-hydroxy D, and you have three intervention groups here. 400 IUs per day, 4000 IUs per day or 10,000 IUs per day, every day for three years. And so but if you look at the baseline 25-hydroxy D levels of those groups, it was 76, 81, and 78. So, all above, anything would expect. So, in that lowest group, the 400 IUs a day, they end the trials still on 77. So, they've maintained this kind of completely adequate slash even optimal, depending on what guidelines you're looking at 25-hydroxy D status. And so then when you see a lack of a change here, then you end up with conclusions of there's no benefit to say vitamin D supplementation, well, there's no benefit in the context of someone with already 25-hydroxy D levels of 75 and above let's say, would be a more fair conclusion. So, it's just something to be aware of, as Alan's pointed out, saying, there's no benefit of vitamin D, it's kind of quite an absurd position, because then you could say, well put someone on zero intake or zero sunshine and get their vitamin D status through the floor. And let's see what happens.

ALAN FLANAGAN: It's an untenable conclusion, because it's a matter of empirical fact that nutrients have biological activity. So, to say there's no effect is just like it's not a correct way of framing a

conclusion, the added context of the hardware how and when so to speak has to be added to that conclusion to make it meaningful.

DANNY LENNON: Right, cool. So, I think that is a good summary of where we're at with vitamin D, we don't need to dive too much further on that. I think maybe to round this out, because there's a lot of other kind of smaller minerals you may could have mentioned, or maybe we might get to some stuff on some phytoestrogens. But I think of the kind of other nutrients we plan to look at was maybe on vitamin K, because there's a couple of really interesting things just for people to note, and it's one that will get brought up because of how it plays a role mechanistically with some of these other nutrients. So, with vitamin K, we have some prospective cohort studies, we can maybe mention that there are some intervention trials, and then maybe also looking at different types of Vitamin K is quite interesting. And I know this is an area you've certainly looked at. So, from an overview, what's the kind of introduction into vitamin K and the bone health literature?

ALAN FLANAGAN: The introduction would be that kind of at the level of understanding of the synergy, the potential synergy between these various kind of topline nutrients that we are interested in for bone health, we've obviously talked calcium and vitamin D. And you can add k into this. And in a really gross oversimplification of these processes, we could think of vitamin D as well, we know it enhances calcium uptake. And then with vitamin K, again, gross oversimplification, but vitamin D is enhancing calcium uptake in the gut, Vitamin K is kind of helping calcium actually get into bone, so to speak. So, Vitamin K is essential cofactor for what are known as vitamin K dependent proteins, VKDPs and they are crucial for bone metabolism. And overall, we don't necessarily need huge amounts of vitamin K, the adequate kind of daily intakes are around 120 micrograms per day for men, and then 90 micrograms a day for women. And

it's with vitamin K, we have K1 and K2. So, K1 phylloquinone are primarily found in greens, spinach, and these kinds of foods that can be converted to K2. From a bone health perspective, there may be an interesting difference, because the half-life of K1 is quite short, which may mean that from a dietary perspective, you'll kind of need a relatively continual intake. And that would be similar to a lot of, say, B vitamins, for example. Vitamin K2 might actually have a much longer kind of half-life and so have a more continual action on these bone formation proteins and Vitamin K dependent proteins.

So, at the kind of at the top line, you know, there's been these associations in prospective studies. And there may be important difference in terms of where these studies are conducted. And we'll talk about that from a Western developed country perspective, we have the two kind of major cohorts that looked at this in the US were the Framingham and Nurses' Health Study, the Nurses' Health Study, huge study, but it is not necessarily representative of the, you know, whole the US population, it's in 98% white Caucasian women, but there was 72,000 followed over 10 years and comparing less than 90 micrograms a day, higher levels. There was a 30% increase in hip fracture risk in people with, so less than 90 is obviously the kind of RDA for women. And then in the Framingham study, they compared the high versus low comparison was less than 56 micrograms per day of K1 versus 250 over 250 and compared to that, so the group consuming over 250, so that's a lot of K1 intake had a 65% lower fracture risk. And then if we kind of go over to Asia, because the Japanese were the first to actually make a pharmaceutical grade with K2 supplement menaquinone, which is MK-4. There's different menaquinones, so there's different numbers at the end of them, that is a reflection of their structure. So, menaquinone-4 and menaquinone-7 are the two primary vitamin K2 derivatives of interest. But in terms of prospective studies, in Japan, there have

been a number of studies that have looked specifically at K2 intake because there's a food source of fermented soybean that's common in the diet and it contains this particular type of fermented bacteria. So, Natto is the name of the fermented soybean dish, but it's specifically rich in MK-7. And there's been associations of high MK-7 intake and lower fracture risk and slower rate of BMD loss, bone mineral density loss, particularly in postmenopausal women.

So, we have these western cohorts have looked at K1. And they've suggested that higher better than lower and that, you know, higher or that certainly low intakes are associated with increased kind of fracture risk, although interestingly, they didn't find an effect on BMD. And then we go over to Japan, which is a country that's an interesting kind of case study for vitamin K, because they have this food source that is rich, specifically in Vitamin K2 as a product of fermentation. And we've seen some of these associations. And then we kind of get into the interventions themselves. There's a number that have looked at K1 and then there's a few more recent that have looked more specifically at K2, again, both M-4 and MK-7.

In relation to vitamin K1 supplementation, the overall kind of conclusion is that the evidence is fairly underwhelming. Sara Booth had a review of the kind of available interventions, now it's a little dated at this point, it's 2009. But there is little evidence of benefit in a wide range of supplementation of K1, you know, up to 5000 micrograms a day, over a period of one to three years. The exception was a study by Raman colleagues, and that was published in 2003. But that study difficult to tease apart because it was looking at postmenopausal women between 50 to 60 years of age, but it had a placebo. And then it had a calcium, magnesium, zinc and vitamin D3 supplement. And then it had the same supplement plus vitamin K1. And the finding of the study was that there was an additional benefit to bone mineral density in the supplement additionally

containing K1. But that doesn't really give us any sort of conclusion in relation to the efficacy of K1 in isolation, because you were adding K1 to a matrix of beneficial nutrients for bone, specifically calcium and D3. So, the question that this trial would leave us with is, is there an independent effect of K1? Is there an effect of K1 absence inclusion with calcium and vitamin D3? And that's kind of been an open question in relation to some of the K1 trials and ultimately, they're relatively underwhelming.

And then for K2, we have the use, kind of fairly established use of synthetic MK-4 in pharmacological doses in Japan, and it's an approved treatment for osteoporosis in Japan, doses for MK-4 commonly about 45 milligrams per day for the most part. And there was a meta-analysis that kind of looked at these trials and reported that, you know, 45 milligrams of MK-4 over time, over six months, you know, significant reduction in fracture risk. And the magnitude of effect was huge, you know, 77% reduction in hip fracture risk and otherwise. But there's kind of limits to this meta-analysis because the inclusion of the primary studies, if you start to pick them apart, they're not really great studies. They're small trials, there's often again, concomitant calcium and vitamin D use. So, the question really is still bagging of whether there's a kind of an independent effect of vitamin K that might be separate to a potential interactive effect with calcium and vitamin D or is perhaps, you know, which was suggested by the Raman colleagues trial.

But we do have a couple of more recent interventions, one last year, one in 2013 that used MK-7 specifically. And what's interesting about these is, as I mentioned with some of the observational research, there is a potential modifying effective menopausal status. And some of the observations have been primarily observed, particularly for K2 in postmenopausal women. And the interesting thing about the kind of more recent interventions, the Knapen and colleagues. It's

K-N-A-P-E-N, 2013 intervention 180 micrograms of MK-7, and then Zhang (ph) and colleagues last year in 90 milligrams of MK-7. And these both showed an effect of, particularly the Knapen and colleagues trial was interesting, because it was over three years, there was a time course evidence of benefit, where after the first year, there was no real difference between the groups. And then they started to diverge, and you start to see a protective effect of the supplemental intervention start to emerge in the second year and was strongest at the conclusion of the intervention at three years. So, suggests potentially a time course effect. The Zhang and colleagues intervention also suggested a benefit in postmenopausal women, similar to that previous study I just mentioned, but it was also a calcium plus vitamin D3 trial that the K2, which was 90 micrograms of MK-7 was added to. And, you know, it suggested ultimately that there was kind of, you know, no additional effects with the calcium and vitamin D. So, overall, well, vitamin K and the difference between K1 and K2 is quite interesting. It's really difficult at this point to isolate independent effects from vitamin K from other bone health nutrients, like calcium and vitamin D.

I think the ultimate conclusion that we could possibly make at this point is that where people have sufficient calcium and D3, that there may be little added benefit of vitamin K, I think that's probably a reasonable conclusion we could make from the evidence at this point. But that's not to say that that's the end of the story. A lot of the trials to date have been small, some of them haven't had a control group. Some of the more recent studies are more suggestive of a potential benefit, specifically of MK-7, and particularly in postmenopausal women. So, there's definitely more research to be done on the kind of more independent effects of vitamin K. I think really these are trials that should probably be conducted isolating the effects of MK-7 supplementation itself independent of

calcium and vitamin D. But overall, for the kind of very mechanistic interest in vitamin D and some of the very suggestive conclusions from some of the cohorts and indeed interventions in Japan, the body of evidence is not as robust as some of the hyperbole would suggest.

DANNY LENNON: With that summary, there's not much of a change. I think there's some interesting parts of that that would apply to a number of different debates or discussion points in this area of vitamin K that I sometimes see get brought up. So, typically, and like you say, there's, there really could do with a lot more research in this area. But there seems to be some general trends around when it's specifically bone health we're talking about it seems to be that K2 is more interesting than K1. That Brahms study which was K1 supplementation had like quite a high dosage as well, not something you get necessarily from diet, but within K2 then we have either from dietary sources, the MK-7 that you're getting from, like fermented soybean seems to be, that could have potential benefit. But there's a difference and that it has better absorption than some of the MK dietary sources specifically. So, some things like meat or dairy, some of the animal based products that people point to as sources of K2 are sources of this MK-4, but it doesn't seem to be as well absorbed from what I've been able to see as some of the MK-7. And so some of the benefit in that area is supplementation with MK-4 like really high levels. But I guess the kind of question at the end then is, do we need to include sources of MK-7 within the diet in the form of Natto or other kind of fermented foods? Not really much to suggest, like you say in an otherwise nutritionally kind of replete diet that someone would need to do that per se. And in relation to the supplementation then, whilst there was some of that kind of MK-7 supplementation being beneficial in postmenopausal women, how that applies to other kind of populations again, I don't really know so, yeah, I think that's an excellent

overview of vitamin K. So, that should do us there.

I think we're close to coming up with time. So maybe just before we finish, like we noted earlier, there's a few other nutrients that sometimes might get brought up, might be a bit interesting, we probably won't dive into them here. So, we've minerals like potassium, magnesium and phosphorus may be interesting to discuss. But one in particular might be useful just to kind of bridge into another topic would be the role of dietary phytoestrogens, because this is one where there has been research looked at, and particularly in relation to production of various functional foods, dietary phytoestrogens have been used, because their hypothesis about them being potentially beneficial for bone health. And this relates to changes in estrogen levels. And we noticed right at the start that these changes in the sex hormone estrogen can have an impact on bone. And this profound change around menopause where we see this dramatic change in estrogen levels. And you see then pretty dramatic changes also in bone in around that kind of two, three-year window perimenopause. So from that, I don't know, if you want to kind of mention more around that particular time in life and how that relates to bone. But then even beyond that, then does that actually play out when we're looking at something like dietary for the estrogens? Is there any evidence that they can actually make up for these changes in estrogen that we see?

ALAN FLANAGAN: Yeah, I mean, there is some evidence of effects on bone mineral contents, potentially, again, modified by people with low VMC at the outset. But there's kind of divergent effects that, again, that there's this difficulty if the vitamin K research is a bit clouded by the fact that calcium and vitamin D is thrown in on top then some of the soy isoflavone supplemental interventions are clouded by the fact that HRT use is often concomitant, although sometimes this has been distinguished. I think the first

RCT, looking specifically at one of these kinds of interventions was a 2009 study that looked at Genistein, which is one of the soy isoflavones specific to soy. And HRT is a hormone replacement therapy. And it was looking again, postmenopausal women and this was a kind of a double blind RCT. And it was looking over 12 months and they looked at bone turnover markers. But also, you know, with bone mineral density. And what was interesting about this trial is that the Genistein supplementation in terms of the percentage increase in bone mineral density in the femur, the femoral neck, the Genistein had slightly more of an effect than HRT, both of which outperformed the placebo, and also with the lumbar spine, HRT had a slightly greater effect. So, you saw a kind of a similar effect on those outcomes, which suggested that kind of positive effect of and perhaps related to that kind of mildly estrogenic effect of these, which are phytoestrogens in relation to HRT. But this is not necessarily a consistent finding. There are contradictory findings within the isoflavone research.

I think an overall summary would be that, you know, there's a mild effects evidence and it could potentially be modified by menopausal status. Certainly, if we're looking at just the bone metabolism or bone turnover factors, there was a systematic review and meta-analysis of RCTs that came out this year looking specifically at soy isoflavone on markers on bone metabolism, and you generally see, you know, some positive association as in positive impact on bone turnover markers. So, there's a beneficial effect on those markers. And the question is, how much does that translate into kind of like, “hard” outcomes in relation to say BMD or even fractures? There was another 2009 study, which was the survival study is the acronym soy isoflavone for reducing bone loss three year RCT, again, postmenopausal women, and that was one of the null findings, didn't show any effect of the extracted soy isoflavone

supplement, which was taken from soy protein. I think overall, what effect is evident in some of the studies is a mild effect at this point,

DANNY LENNON: Like we had mentioned there are others we could have discussed, but in general but not as important or as contrary amount of evidences we have for the nutrients we have discussed so far. So, maybe another time we can dive into some of those other minerals. I think maybe just finish up, there might be a worthwhile just mentioning something about kind of public health nutrition guidelines from this point on, given, we've talked about this the burden of this issue at a population level and how big of an issue it is. And given our discussion of some of these trials, where we can see actually some decent differences in that kind of older demographic, people in residential care, who are probably at the highest risk of some of these fractures. And while that's obviously great to have, I guess, from a public health level, one of the things may be worth discussing is that avoiding fractures in a relatively small number of people at high risk. So, small number in terms of relative to the whole population, maybe isn't really reducing that burden that we're seeing at fractures in the community in that, that population burden of fractures. And so number events, we're seeing in the morbidity from that the economic cost that we mentioned to the community is arising in a lot of like, large numbers of people who are not at that super high risk level, they're actually quite a moderate risk. And so because we're seeing that, that kind of leads us in a kind of sticky place when we look at some of the interventions we've discussed, and their actual applicability to what to do from a public health level, if that makes any sense. So, I don't know kind of what your kind of thoughts are in this area? And with current attempts to try and improve bone health at a population wide level? And you're taken up?

ALAN FLANAGAN: Yeah, it's difficult, because, you know, like, Vitamin D is a fortified nutrient. And food

fortification programs, obviously, face the challenge of trying to balance, you know, the levels that are going to be put into the food supply so to speak with potential levels that people already have, and all of that, and that's largely kind of not necessarily worried about deficiency, because obviously, the purpose of food fortification is to prevent that. But it's more worried about, you know, if people have excess intake. And indeed, we do see that when you look at the effect of fortification or even people taking multivitamin supplements. And you can see that there are some nutrients of which there are levels of intake in the population above what would be considered the tolerable upper limit. Now, it tends to be for B vitamins, which are, you know, high turnover rate. And, you know, whether there's a kind of any, the adverse effect of that is unclear. And I think, you know, for vitamin D, there's still this debate that rages about what these optimal levels are and what would be desired to be achieved, you know, in the population. There does seem to be obviously a growing use amongst, shall we say, people, like middle class healthy people tend to be ones who will actively take supplements, that's fairly consistent, higher socioeconomic status, higher education status, all correlates with the vitamin use.

I think, if we're factoring in some of the say, the calcium research, is there something there that kind of, perhaps the focus, because this is something that obviously like any of these disease outcomes stretches over the course of the lifespan? So, you know, is there a place that the emphasis perhaps need to change? There's obviously a big emphasis now on the mechanical loading aspect of bone health and recommendations in the elderly to remain in some sort of active state and weight bearing exercise. So, I think those recommendations seem to be more widespread. From a purely nutrition perspective, I'm not sure for us necessarily would change in terms of current advice that would make some sort of massive

shift or even population shift to a lower kind of risk category between our current understanding of nutrient requirements and current recommendations. I think that the gap is obviously the implementation, probably at the population level, I think a reasonable interpretation of the evidence would be that it's vitamin C and sufficiency is probably the primary concern from a bone health perspective, particularly in countries like this, where people are eating their yogurt and drinking milk, you know, most people are at least certainly in adulthood, likely have adequate calcium intake.

So, I think vitamin D status is the primary concern. And, you know, there, despite food fortification, despite people using supplements, the prevalence of vitamin D insufficiency is still fairly widespread in the population. So, that would appear to be the lowest hanging fruit. Now how that's addressed, whether it's public health specific recommendations for dosage ranges or, you know, increased widespread fortification, right now it's primarily milk, whether food fortification becomes more widespread in different foods, all of these avenues might need to be explored or considered in order to maintain sufficient levels on a more year round basis, because there is that seasonal cyclical nature of vitamin D levels, which may of course be or is hypothesized to be one of the factors that contributes over time to these kinds of adverse bone health outcome. So, I think the lowest hanging fruit would be maintaining, achieving and maintaining adequate vitamin D levels across the whole population, what vehicles are used to achieve that, I think it's still something that will be up for kind of debate within public health.

DANNY LENNON: Yeah. And then so from an individual level, then, I guess for people, there's probably nothing too specific other than saying, maintain a total calcium intake, that would be kind of in line with kind of recommended

guidelines. Same as we discussed with vitamin D, something that is not an insufficient level, and make sure maintaining good levels of that and overall healthy dietary pattern that's probably not going to leave them massively had deficient in any other potential nutrient of concern. But certainly with calcium and vitamin D in particular, and doing that from a nutrition side, you're probably doing all you can, and that can be kind of consolidated with the fact that we have these now findings from certain RCTs by virtue of people within those studies have adequate levels, say of vitamin D, or are kind of calcium replete, and so aren't going to benefit from additional intake, because as you've noted, it's not more is better, it's can you achieve this certain appropriate level of intake for these nutrients, and that's probably going to support bone health as best as possible in line with all those other factors both modifiable and non-modifiable, that will lead to an individual's kind of risk of osteopenia, osteoporosis, they'll be kind of the things to note.

ALAN FLANAGAN: Yeah, I think for these latitudes, you know, assuming that most listeners, I think are probably physically active and, you know, have a good dietary pattern. Probably the lowest hanging fruit would be, you know, vitamin D supplementation in the winter months, particularly if they're not getting outdoor light exposure or sun exposure, you know, between I think it's October to March is generally the kind of window that it's recommended. And that's probably the one thing that people could do if they're not doing it currently that would kind of make the biggest benefit.

DANNY LENNON: Cool. With that. I guess we will wrap it up there if you're happy as an end point.

ALAN FLANAGAN: Yeah. I couldn't find any boneflex. Yeah. Look, if anyone out there is looking for an avenue for to write a book or start a whole blog or generally get into nutritional quackery, I highly

recommend bone nutrients and bone nutrition. It seems to be really just ripe for the taking.

DANNY LENNON: Well, I do know Mr. Saladino has a supplement line, something called “bone matrix”.

ALAN FLANAGAN: Doe he?

DANNY LENNON: He's already on the bone health train!

ALAN FLANAGAN: It needs to be called “the bone myth” or “the bones solution”. And it needs obviously to include ancestrally consistent recommendations for bone health.

DANNY LENNON: Okay, excellent. We will wrap it up there. Thank you, everyone, for listening into the episode. Again, we very, very much appreciate it. Please continue to share word of the podcast around, let other people know if you think it might be useful. Continue to send us your feedback and questions. It's all very much appreciated. Myself and Alan, we’ll be back in another episode very soon. We've got some exciting stuff coming up, which we're going to make more announcements about in coming episodes. So, look forward to that. We'll put show notes this episode over at sigmanutrition.com/episode411. I will link up to all the studies that we mentioned throughout this episode. So, you can go and look through the details of those. I'll put up some supplementary information that might be useful. I will link to Alan's recently published paper, and then anything else that might be relevant to help you kind of learn and retain some of the information we've covered here. And with that, please tune in for our upcoming episodes. And yeah, thank you for listening. And till next time, I hope you stay safe and take care.