



Danny Lennon: Hello and welcome to another episode of Sigma Nutrition radio. This is episode 444 of the podcast. My name is Danny Lennon and I'm here with Alan Flanagan. How are you sir?

Alan Flanagan: I am very good. Thank you. I'm now no longer just a candidate for a PhD. So it's nice to be over the line.

Danny Lennon: Yeah. A big congratulations are in order a big congratulations. And we'll certainly maybe touch on some of what came out of that PhD and some of your publications at our later time point. But for today we're gonna be talking all about folate and there's a number of interesting avenues for us to explore throughout this conversation. We're gonna be talking about folate some of the metabolism of that how it's involved in various processes in the body we'll be looking at folate deficiency and then some of its impacts on various disease outcomes.

We're talk about folate really. It's actually maybe a we can think of it as a collection of these natural folate that we're gonna get in food of different types that we'll mention. And there's also synthetic folate. The most obvious of which that we're gonna discuss is folic acid. but there are other forms for example folic acid and folate is often.

Refer to also as vitamin B9. but as we are gonna discuss there are different forms of folate. And we're gonna try and distinguish between those from food sources. We're typically talking about this coming from things like green leafy vegetables whereas a large proportion of folate in the average person's diet will come from fortified foods.

And of course they are fortified with this synthetic form of folic acid. Now one of the big areas that we've discussed recently in the last episode of the podcast that we've discussed

is this idea of one carbon metabolism. And this's something we're gonna revisit today. And so if people either haven't heard that episode or as a brief recap I think it's useful to explain what we're talking about with one carbon metabolism looking at the folate cycle looking at methylation and some of the.

Key aspects of that that actually will be important to understand the rest of this discussion. So maybe I'm gonna hand it over to you to start us off. Alan what is the kind of initial introduction that we could give around one carbon metabolism the methylation cycle and then specifically folate and its role within that.

Alan Flanagan: So you mentioned one carbon metabolism. So this is basically metabolism of groups in the body compounds in the body that have exactly that they have one carbon there's a single carbon atom with other chemical elements that will make up a particular compound or group one of which is known as a methyl group.

So a methyl group is again a one carbon atom. So it's related to this concept of one carbon metabolism. and it's bonded with three hydrogen atoms. This together is what like I said we would call a methyl group. So in this concept and this can be referred to as the cycle that we're going to work through now could be referred to as the methylation cycle or again this concept of one carbon metabolism.

What happens with this process of metabolism is the process of adding. A methyl group to another preexisting molecule. And the addition of that one carbon methyl group to another molecule can either then inhibit or initiate reactions that occur. And so this process of one carbon metabolism is quite important for a number of processes the repairing of DNA the regulation of genetic and epigenetic expression and processes and the regulation of the processing of two important amino acid.

homocysteine and methionine and cystine itself it's breakdown into those component parts the actual cycle itself. there's a donor group. That's responsible for this overall process of transferring one carbon methyl groups to other molecules. And that's known as S-adenosylmethionine or SAM-e.

And the process of producing this donor is known as the methylation cycle and homocysteine is produced as a byproduct. So this cycle begins with what is known as five methyl-tetrahydrofolate. And this is the active form of dietary folate. What we would know is vitamin B9 and this step itself requires the function of an enzyme known as methyltetrahydrofolate reductase or MTHFR.

And it's through that enzyme that folate. Enters into this cycle. And what we end up having is a series of donations of these one carbon groups result in the cycle ultimately producing homocysteine . There are a number of co-factors all of them B vitamins that are important for this cycle to function.

And in order importantly to recycle the homocysteine back into the amino acids methionine and those would be in addition to folate which is our focus today vitamins B2 riboflavin vitamin B6 Perine and vitamin B12 or Cobalamin. But of these could be distinguished into some form of hierarchy and folate appears to for the purposes of this folate cycle this process of methylation and the recycling of homocysteine appears to be the most important secondarily along with B12 and then B6 appears also to be important. but there is evidence for example that the magnitude of homocysteine lowering will be more dependent on folate. And there's some research we can discuss down the line in relation to that.

So this cycle is highly important. Like we said for kind of genetic and epigenetic expression for DNA function for regulating amino acid and their role in the production of homocysteine . And they're recycling into methionine and cysteine. And the entry into this cycle is an enzyme through which folate passes. The functioning of that enzyme is something we'll discuss in relation to some important genetic variants in folate metabolism and folate itself is a primary cofactor in the appropriate functioning of this cycle. The appropriate functioning of this cycle of which also relates to the ability to metabolize homocysteine to methionine and cysteine. And in turn reducing the levels of homocysteine which is an implicated risk factor in a number of the disease endpoints that we'll discuss.

Danny Lennon: Maybe let me try and recap. We have this one carbon metabolism which is referring to these compounds with one carbon of note here. You mentioned this folate cycle which we can think about as having the source of that folate from the diet is gonna be folate or folic acid. The folate then is acted upon a certain enzyme dihydrofolate reductase converts it into another form dihydrofolate itself. So DHF. That same enzyme then can act on it again converts it into Tetrahydrofolate which is something people may have heard come up in this conversation.

I'm sure we'll mention a bit later on this. In turn can get converted into five 10 methylene-THF. And then the enzyme that is of particular note that you outlined was MTHFR. And we'll mention that particularly in relation to some of the genetics later that enzyme acts on this five 10 methylene THF to convert it to five methylene-tetrahydrofolate.

And there's another of other steps that include homocysteine and methionine some of those enzymes involved like methionine synthase relies on things like vitamin B12 the roles of other B vitamins here which are important. So with that as our kind of. I suppose initial starting point maybe we can start to talk about from a health perspective what happens when we don't have appropriate amounts of folate. And so we can think about okay what happens in cases of folate deficiency but maybe before that we might need to work out well what is folate deficiency? How do we think about folate status?

Alan Flanagan: In terms of deficiency like any nutrient that we have in the diet most important concern would be dietary insufficiency. We know that folate deficiency can occur with other factors for example heavy alcohol intake and otherwise and as a water

soluble vitamin with a quite a high turnover rate it's important that actually there's a fairly constant intake of folate or folic acid. And there are a number of clinical deficiencies; the most important of which would be megaloblastic anemia. And so with megaloblastic anemia it's where you have a low red blood cell count and there are other morphological abnormalities associated with the cells themselves the kind of the precursor cells to red blood cells.

And so this type of anemia is one of the main concerns for folate deficiency. There's an indication in that itself as far as what we look at for red blood cell for folate status. So I mentioned megaloblastic anemia was characterized as a low red blood cell count when looking at folate deficiency.

Red blood cell folate is the best biomarker for folate status in the body. There's a number of reasons for this generally red blood cell biomarkers overall are a reflection of because of the turnover time of red blood cells of 90 to 120 days. There are also a biomarker of dietary take over that core over that more shall we say medium term as opposed to plasma status?

,particularly for a nutrient like folate which again has quite a high turnover rate and plasma's not a reliable biomarker for folate status. As a result plasma at best would be an indication of very recent folate intake but because plasma concentrations respond so rapidly to changes in dietary intake it's not a reliable measure of more stable folate status.

So that would be why red blood cell folate status is preferable is there's been some suggestions that you can actually use normal blood homocysteine concentrations. Again as this we just discussed as the indicator of one carbon metabolism that would be a proxy for adequate folate intake but that's a very ancillary indicator like that.

That would not be something that would be ideal or preferable. So in terms of defining deficiency in relation to red blood cell folate status that would be levels of less than 305 nanomol per liter would be indicative of inadequate folate status. And then in terms of thinking about. Achieving adequate folate levels.

It's important to understand that there can be bioavailability differences between foods and supplements. As an example the type of folate that naturally occurs in foods are quite structurally unstable and they're influenced by exposure to heat. For example with cooking or even just storage fresh vegetables can lose over 50% of their folate content in being stored for say three days or over the folic acid that's used either as a supplement or in food. Fortification is a more chemically stable. Ultimately as a result there are differences in the they have comparable bioavailability and absorption at the same dose but the actual kind of levels of what you would get out of it as far as contributing to adequate folate status are slightly different.

And so for this reason there's this measurement that has been developed known as dietary folate equivalents or DFE. And that this typically represents the fact that you would get a higher bio availability of synthetic folate acid because of the stability because of the greater stability of the compound.

One microgram of food folate is equivalent to one microgram. Of dietary folate equivalents. But like we said there is a bioavailability factor that in foods that relates to the kind of instability of the food based compound. And so if you have one microgram of folic acid either taken as a supplement or in fortified foods that would count for 1.7 micrograms of dietary folate equivalents.

So if you had a meal for example that had a hundred micrograms of food folate and ,you took that as folic acid for example. that would be equivalent to 170 grams dietary folate equivalents. and there's evidence of perhaps higher bio availability higher contribution to dietary folate equivalents.

If folic acid supplementation is taken on an empty stomach again all of this then contributes to the maintenance of adequate folate status in the population where we factor in a combination of both dietary intake and fortification through foods and even potentially people taking multivitamins importantly since the introduction of mandatory folate fortification in certain countries which have undertaken that there's no evidence of additional folic acid having any risk of kind of toxicity or otherwise in humans.

So the combination of food availability plus fortification in foods In addition to individuals in the population who may in fact supplement on top of that there's no apparent risk of that. For example from a population health perspective which is a really important consideration whenever food fortification is being undertaken and the countries that have introduced food fortification have largely shown benefits over time particularly to outcomes like adverse pregnancy-related outcomes

Danny Lennon: With folate deficiency you outline that there's these a number of different both genetic and lifestyle factors that can lead to this or at least increase risk of a folate deficiency. so you mentioned things like chronic heavy drinking smoking. We could look at the role of certain medications. There's been evidence around certain malaborpative conditions like IBD or Coeliac disease, cancer, inflammation. Pregnancy is a time where folate needs are increased.

So all of these things could play a role but what we'll probably get onto a bit later is looking at the role of dietary insufficiency in particular. And within that you gave the example of megaloblastic anemia which can result from either deficiency of folate or vitamin B12. This is where we have a dietary insufficiency of enough folate then causing in this case an anemia but there are other issues that we'll maybe touch back on in relation to chronic disease.

Within that though there. Often something that comes up that people I'm sure may have seen online. And I think oftentimes it actually comes from maybe I dunno functional medicine type circles where there's this actual pushback against using folic acid to any degree whatsoever. And in saying so well you want to get folate and not only dietary folate if you're gonna need to supplement maybe go for one of these like Tetra hydro folate forms but certainly don't go with synthetic folic acid because there can be some issues.

Now there is maybe some basis for looking at this in the context of what you mentioned earlier around maybe potentially masking an issue and so on or that there is gonna be differences in how they're metabolized but what is your kind of sense of where these arguments against folic acid stem from?

Alan Flanagan: I don't know whether perhaps more of the kind of nature fallacy at play. The typical argument that you'll see is that wild is: five-methyl-THF bioequivalent supplement essentially that that is in the form of dietary folate would be preferable to folic acid. But in terms of the evidence both the kind of supplemental dietary form of five-methyl-THF and folic acid appear to have.

Comparable bio availability comparable absorption comparable effects in the body at a dose for dose comparison. the only potential advantage is something that you've alluded to which is there is a suggestion that the use of the five-methyl-THF form would not have the same potential to mask the symptoms of vitamin B12 deficiency that could occur with folic acid.

So one of the things we referred to megaloblastic anemia right? So it's a this type of anemia where you not just get the impact on this like insufficient red blood cells but also their enlarged red blood cells. and this can be caused by both vitamin B12 and folate deficiency and a very high intake of synthetic folic acid can potentially mask deficiency in B12.

So if the cause of the megaloblastic anemia is vitamin B12 deficiency that may be masked by folic acid high intakes of folic acid supplementation. This is generally something that is navigated by. Administering both folic acid and B12. in the context of acute cases of megaloblastic anemia the complexities of this are exactly why you would want an appropriate nutrition professional do going through this with someone rather than some of the more kind of quacky approaches which are like not overseen and probably not evidence based. So yeah as far as I can see I don't think at any kind of evidence based level there's an reason to draw a massive distinction between folic acid and the kind of supplemental versions of five-methyl-THF.

Danny Lennon: One of the other... or just a an area where there is a lot of conversation and maybe a lot of conflicting information that people come across is in anything relation to genetic variants because this is somewhere where we have clear evidence of a wide variation in genetics related to folate metabolism.

So particularly looking at certain genes you mentioned that the gene for MTHFR; so this enzyme that plays an important role and due to certain variations we can see that does indeed impact folate status. However that then leads to no shortage of places. People have seen online where. the most important thing you need to do for your health is get a genetic test.

And then if you have these certain types of genotypes you need to go high dose supplementation on these specific types of folate or all different types of kind of claims here. So there's obviously a lot of interest in this area. So maybe if we start trying to walk through what do we actually know about these genetic variants?

,as I've just mentioned that relates mainly to MTHFR can you maybe give an overview of what you think are the main things you have taken from this area of literature?

Alan Flanagan: So at the start we discussed the concept of one carbon metabolism and both the kind of the folate aspect of the cycle and the overall methylation cycle as well. And the reduction in homocysteine. And we mentioned that shall we say the kind of the gatekeeper to that cycle is this particular enzyme this methyl Tetra hydro folate reductase or MTHFR. and so the functioning of that enzyme is critical to the entry of folate in into that cycle and all of the processes that that we discussed and outlined in discussing those cycles and their importance to physiological function.

There are two main common genetic variants. there are a lot more I think there's more than 40 single nucleotide polymorphisms in the MTHFR gene but two mutations in particular. Have been the subject of the of most study. And that's because there's actually a fairly high prevalence of some of these polymorphisms in the population.

So the two genes in particular the mutations are 677C or 677T. And there can be two forms of that. someone can be homozygous which means that they've it's 677 TT. And then you could be heterozygous which is 677 CT. So it's where the it's where the C is replaced by a T in this genotype the importance of these variants is that the enzyme that we mentioned under functions and the activity of the enzyme if you have the heterozygous type; so if it's 677 CT, that enzyme under functions by about 30%. And if it's homozygous so 677 TT then it under functions by about 65 to 70% compared to the homozygous CC genotype. So in effect this means that less folate is going to get into that cycle. That cycle is not going to run as it should.

And there will be factors or outcomes like increased homocysteine level actual reduced folate status and potentially increased risk for as potentially a function of the increase in homocysteine levels. either neurodegenerative disease or cardiovascular disease. Now there is evidence that all is not lost.

So to speak supplementation with folic acid essentially. Provides the means to overcome the under-functioning of those genetic mutations. There was a paper in 2003 by Guyenot

and colleagues which looked specifically at the intake of 400 micrograms of dietary folate equivalents which in both genotypes that I described.

So regardless of the genotype whether it was homozygous or heterozygous the repletion with 400 micrograms a day of dietary folate equivalents normalized blood folate levels and also brought plasma homocysteine concentrations into more desirable ranges. There is another genotype as well which is a 1298C but the reduction in the activity of that polymorphism is generally related to lower bio availability of folate in cells. It's not as much of a focus as the 677CT genotype.

Danny Lennon: So we see these changes in or this certain polymorphism come up where as you noted in this MTHFR C677 there's a change from a C to a T that can either be homozygous or heterozygous. And then that leads to reduced folate status and things like increased homocysteine concentrations which then in turn can lay a foundation for a number of the negative health outcomes that we're gonna touch on now in a moment. So in relation to maybe some of those disease outcomes maybe you can start walking through them. Some of these have come up on recent podcast episodes. People will have heard on a couple of the recent episodes around pregnancy discussions around folate's role in say neural tube defects which we can certainly dive into and certainly dive into maybe some of the interventions and trials there.

But there are then a number of other disease outcomes which are worth talking about cardiovascular disease. We've mentioned already neurodegenerative disease you've just mentioned and then potentially even cancer. So let's start with the most clear cut or at least I think the one that most people tend to be familiar with when it comes to folate and that's its role in pregnancy. And particularly first of all in relation to neural tube defects.

Alan Flanagan: Yeah. So neural tube defects are a defect of the closure of the spine essentially. And so when the spinal column is forming in the absence of adequate dietary folate and again the period of repletion of folate is important in this regard because this process occurs during the first trimester.

So it's in that prenatal period that repletion with folate it is important essentially a part of the spine will form and fuse outside of the body. This is something that in the early analyses and even in the kind of early trials that were born out of these analyses it was noted that there was a particular temporal relationship between adequate repletion.

,adequate folate status or higher compared to lower folate intake and the prevalence of this really debilitating and unfortunate outcome. and so that particular temporal relationship was identified. and we can see some similarities there with other nutrients of interest during pregnancy period like iodine for example. So in relation to folic acid supplementation or just folate repletion it became obvious that actually it was in the period prior to conception and indeed the first trimester and that for example intake after that wasn't necessarily going to have the same impact on lowering risk of this outcome.

It was also recognized that folate concentration we discussed folate status when we were talking about deficiency but the concentration required for pregnancy is much higher. It's possibly difficult to achieve just through diet alone. For context we said earlier that a deficiency could be defined as a red blood cell folate status of less than 305 nano mil per liter ideally for pregnancy related outcomes that would be over 900 nano mil per liter. So it was basically undertaken largely based on neural tube defect research that fortification of the food supply with folic acid was going to be a factor that could contribute to increasing folate status in the population and reducing risk of this outcome.

Folic acid fortification is mandated now in 87 countries across the globe. and the policy has been associated with a significant reduction of prevalence in neural tube defects in these countries. Now it's not mandatory in Europe or the UK but it is mandatory in the states and a range of other countries but it's not just temporal observational associations and otherwise that are relied on as a result of those observations. There's being a range of randomized control trials summarized in a recent or relatively recent meta analysis in 2015 of folate supplementation for the prevention of well birth related defects beyond just neural tube defects. But specifically in relation to neural tube defects there was a 70% on average reduction in incidence.

And depending on the trial you look at that can even range from kind of 50 right up to a hundred percent prevention of the of that outcome. So on average the randomized control trials clearly show a quite profound reduction in risk for neural tube defects in supplementation in the population as impressive as this evidence would be in terms of the strength of the effect there remain barriers in the population.

There's evidence that people still are largely unaware of the importance of additional folic acid supplementation in the periconceptual period. yes certainly in relation to this outcome the evidence is overwhelming that this is a causal relationship but it really should be supplementation in the three months prior to conception to achieve the kind of adequate folate concentrations required for pregnancy

Danny Lennon: Right. Yeah. So that's a key thing in this periconceptual period that intervening too late can be a problem. And so making sure that is done early enough. And when we look at those trials that you mentioned particularly these randomized control trials spanning anywhere from 50% to a hundred percent reductions in these neural tube defects.

And that meta analysis that I think you mentioned is interesting that that number that kind of 70% protective effect lines up with I think one of the very first maybe the first randomized control trials that was done specifically on this question the one that was in the Lancet; the Medical Research Council (MRC) study within that trial.

The combination of those two groups that were consuming folic acid you see a 72% protective effect. And then there's other trials where you see again zero cases of neural tube defects. There there are probably other pregnancy outcomes we can talk about

without maybe having to get into the same degree of detail. There seems to be some indications around things like low birth weight with folate insufficiency levels of homocysteine that can then cause issues things like preeclampsia maybe premature delivery might have some relationship there of other outcomes that you may have seen that could be related to folate intake during pregnancy. Is there any that are worth highlighting?

Alan Flanagan: Yeah. I think the evidence after neural tube defect does get a little more inconsistent for preeclampsia. The kind of evidence from interventions suggests that ongoing supplementation beyond the first trimester doesn't really have any significant reduction in risk.

Again there may be some design considerations with some of these trials in terms of the timing of initiation of supplementation or otherwise but overall the preeclampsia interventions don't seem to be overly positive in favor of supplementation specifically congenital heart defects. There's some evidence of ,a modest.

Risk reduction about an 18% lower odds. It's an odds ratio not a relative risk for congenital heart dis defects with again periconceptual folic acid supplementation birth weight. There was a meta analysis of eight RCTs found a modest positive association between folic acid supplementation.

I think each hundred micrograms higher associated with a 2% higher birth weight but no effect on gestational age. So yeah there there are some other potential benefits to pregnancy related outcomes but the evidence is nowhere near as convincing as it will be for neural tube defects.

Danny Lennon: Yeah. And I think particularly on the preeclampsia one of the trials that had a lot of hope maybe before, was the FACT trial which was published a few years ago which was like four milligrams a day of folic acid. And that was one of those that that failed to show really any benefit. If we move on to maybe one of the next big outcomes and this is where that relationship between folate and homocysteine that you outlined earlier becomes really important because homocysteine is one of the big considerations that people have in relation to cardiovascular disease. So before we talk about any of the folate specific literature here can you just remind people again of that connection with homocysteine and cardiovascular disease?

Alan Flanagan: Yeah. So it's been a big kind of topic of debate in medical sciences and cardiovascular sciences in relation to the status of homocysteine as a risk factor much of that appears to be go rounded in trying to tease out whether it's a causal risk factor or not. And harking back to the episode with professor Chris Packard a biomarker doesn't necessarily have to be causal to be important. And I think that's where some of the debate with homocysteine seems to get bogged down. So for cardiovascular health homocysteine yes it has been somewhat of an enigma in epidemiology.

Elevated homocysteine is a consistent and independent risk factor for cardiovascular disease specifically stroke but cardiovascular disease generally. It's generally been unclear by what mechanisms homocysteine might actually which again feeds into its status as a biomarker. It's not something like LDL where we're able to quite precisely understand what it's doing and why.

,it tends to be implicated in damage to the vascular system perhaps exacerbating arterial calcification and other kind of vascular and intravascular related processes. And the other thing that was a knock on homocysteine relates to what we're discussing today which was folate because while B vitamins and folate in particular will reliably in fairly predictably lower homocysteine levels that not necessarily hasn't always translated into that that lowering of homocysteine deliberately by use of B vitamins translates into a lower risk of cardiovascular disease events.

Again there may be some issues with. some of the some of the nutrition specific challenges to RCTs that we've discussed before in relation to other compounds like vitamin E might very well apply here as well. The evidence the reality is for homocysteine is it a causal biomarker? Absolutely not. We can't, based on the lack of specificity for its understanding say that it's a causal biomarker. It's consistently associated with higher levels consistently associated with cardiovascular disease outcomes. And so the question is it a biomarker that is more in that kind of reporter or status or systems biomarker status that professor Chris Packard classified. i.e. Is it something more akin in its biomarker status to inflammation like C-reactive protein for example which is also independent consistent risk factor for cardiovascular disease in epidemiology. And there are deleterious effects associated with homocysteine . So that's homocysteine as a biomarker it's likely not a causal risk factor.

And we cannot say that on the basis of the evidence now but it may be a reporter biomarker that is providing at least some information as to the overall risk profile. The treatment; if we're talking about the use of folate to treat the risk factor Not necessarily looking at cardiovascular outcomes that's pretty over pretty overwhelmingly positive for dietary folate.

There was the homocysteine lowering trialist collaboration meta-analysis which deliberately I looked at pretreatment plasma homocysteine levels of 12 micro Mol per liter which will be high at that's range where you tend to see in the epidemiology pretty significant risk for cardiovascular and neurodegenerative disease but we'll come onto that and do course and basically there was a dose dependent response in lowering homocysteine levels.

So if you had 200 micrograms of folic acid a day it lowered homocysteine by 13% again from this high baseline of 12 micro per 400 micrograms lowered by 20%; 800 by 23%; so did 1200 and 5,000 micrograms of five milligrams a day lowered homocysteine by 25%. Now in comparison the addition of vitamin B 12 to that only provided an additional 7% reduction and B6 had no significant effect.

So this is coming back to these three co-factor. We mentioned at the start and the context of the methylation cycle and the adequate recycling of homocysteine back into methionine assisting. So we do see a dose response relationship between higher levels of folic acid supplementation and the lowering of this risk factor homocysteine .

So the question is does it reduce event risk? and that's where. The evidence is slightly less clear although I think we can parse things together a little bit. So there's been a couple of meta analysis of intervention trials. There was one 14 trials. this was trials published up to 2012. This was B vitamin supplementation not necessarily isolating folate necessarily.,and there was a modest 7% reduction of stroke risk observed in that trial. What was interesting was when they factored in stratifying countries the studies conducted in countries with or without folate fortification policies. And what they found was actually the effect. There was a larger effect in countries without fortification.

And then there was a further trial which a meta analysis of trials which also looked at whether there was an effect mediated by whether those the countries had fate fortification or not. And that showed at a 12% lower risk of stroke where there was no fortification of folate in place. So it may be and this comes back to a principle we talk about all the time in relation to nutrition interventions. If you're starting point of participants on the bell curve of nutrient action is everyone's already within a range of adequacy. It may be that just giving them more doesn't necessarily lead to any better outcomes particularly if events are the outcome.

Yeah. It might slightly have an impact on an intermediate risk factor but it might not actually be influencing pathology that much. But if you've got people who are more towards an insufficiency the addition of that and the bolstering of that status maybe more expected to produce a benefit.

And that's what some of these studies that actually stratify based on fortification suggest. But the implication then is conducting trials potentially in countries with adequate folate folic acid in the food supply. maybe everyone's actually already at a level of benefit and for other kind of cardiovascular events or coronary heart disease events there's another more recent met analysis of 17 trials but these were people with preexisting cardiovascular or kidney disease.

„and didn't show anything any difference between groups and it didn't show a difference in countries with or without fortification. So there's a couple of factors here. there's baseline nutrition status folate status in your population. There's the health status of participants in the trial and then potentially adjuvant therapies as well.

And a lot of these intervention trials there was significant polypharmacotherapy, particularly in the and „,it's likely that there was a lot of masking of any particular there was no isolated effect of folate in any certainly any of the trials that used populations with existing cerebrovascular cardiovascular or kidney disease.

So there's been a couple of more recent studies that might shed a bit of light. One was a recent Mendelian randomization study. Mendelian randomization has a lot of additional assumptions I think for nutrition that make it not as simple and immediately causal as it's sometimes made out.

But for this particular question it's slightly different because one there is quite robust genetic associations for influences on homocysteine. And as we've discussed earlier there are actually really robust genetic associations for folate status. We potentially don't have for some other nutrients.

And what this genetic analysis found was that yes the genetically predicted higher levels of homocysteine levels were associated with 11% increased risk for stroke and then subdividing total stroke that was actually higher for sub OID hemorrhage. But then genetically predicted higher folate status was associated with a 12% lower risk of coronary artery disease and a 14% lower risk of stroke.

And so if we do parse the evidence here we may be able to suggest that if there is an effect or benefit for cardiovascular disease it may appear to be more consistent across lines of evidence for stroke both in terms of observational research intervention trials and this Mendelian randomization study there are those mediating factors that we discussed.

There is evidence of effects on intermediate risk factors which I think warrant folate not being thrown out. And in particular is the reduction in coronary intimate media thickness right? So the thickness of the heart itself essentially in terms of the ,the kind of plaque within it and this was a meta analysis of 10 intervention trials that looked specifically at reduction in CIMT as its outcomes and folic acid supplementation was associated with a significantly reduced progression of CIMT.

And again that effect what was quite interesting in this was that the most beneficial effect was observed. The largest effect size was people either with higher baseline CIMT. Levels of over 0.8 of a millimeter but in those with a homocysteine concentration where the reduction in homocysteine concentration was over 30% from folic acid supplementation that showed the greatest reduction in CIMT like 0.2 two millimeters.

So this was also observed in people who were higher risk cardiovascular disease patients. so there's some evidence of important intermediate risk factors like that being improved that may relate to the magnitude of homocysteine lowering and overall although homocysteine may not necessarily be a causal factor in this it appears to be a biomarker of some importance and status and the evidence from some of these interventions and the MR, does suggest a relationship between the magnitude of homocysteine lowering and reductions in some cardiovascular clinical endpoints of which the most consistent evidence would be stroke. So it's not obviously because of how much has gone into that answer. it's clearly not a clear cut thing where we can say yes folate reduces risk of cardiovascular disease. Like a lot of exposure in nutrition were left trying to dig into and part rather than just say oh it's inconsistent. And that's because nutrition's... doesn't tell

us anything and we know nothing. It's about how can we really dig into this to try and piece things out. So I wouldn't be willing to toss away either the validity or utility of homocysteine as a marker just yet.

And I certainly wouldn't be willing to say that folate or folic acid supplementation is not of some benefit. It may just be in specific contexts particularly where there's maybe an insufficiency of folate in the population.

Danny Lennon: Okay so based on all that there's a few things that maybe we can say first of all that in relation to homocysteine and cardiovascular disease risk there is still some ongoing debate but we can probably at least acknowledge that this is a biomarker of some degree of importance.

We then have pretty consistent evidence around folate being able to reduce homocysteine levels in cases where homocysteine is elevated. We also then maybe have some kind of observations where people with a very low folate intake maybe have an association with higher risk but then when it comes down to actually seeing the impact of folate or folic acid supplementation in trials there's a real lack of being able to show that has a demonstrated effect on hard endpoints of cardiovascular disease.

However there is some other piece of evidence like you said we can maybe piece together that look at other biomarkers whether that's C IMT and looking at atherosclerosis progression could be a benefit there for people at high risk. there could be things more related to stroke or endothelial dysfunction. And certainly then we can come back to the homocysteine picture that we know it it can reduce that but overall we're still left with a lot of unknowns but certainly. Not at the other end to say that oh folate is irrelevant.

Alan Flanagan: And if there is an end point that seems to more consistently pop up across the various lines of evidence it appears to be stroke.

Danny Lennon: So with that maybe let's move on. If we talk briefly a bit about cancer. This has been something where again we have these links between low folate status and maybe increased cancer risk. But again whether that tallies up with intervention trials looking at doses of folic acid particularly might be another question. So again I think probably in a very similar fashion we're gonna have to see okay what do we know across these different lines of evidence? What are we seeing suggestive of that? And as as we have to always know anytime we talk about cancer this is really becomes more complex because it probably differs by cancer type.

And then even within cancer type there are other nuances with within that. So if we do need at any point distinguish between different types of cancers where there's more or less evidence we will do. ,and we can talk maybe first at a kind of overview level. What are the main things that kind of have struck you from the cancer literature in relation to folate intake

Alan Flanagan: Coming back to the concept of fortification and the considerations that always have to go into fortification, in fact one of the suggestions that or one of the concerns in relation to folic acid fortification in the food supply was that if folic acid if folate generally is involved in DNA and RNA synthesis and repair and cell work if there's excess folate could this actually be something that fuels cell and tumor growth?

Again, you could look at some kind of potential mechanistic or cell culture evidence in support but ultimately, I think the first thing to clear outta the way is that there's no evidence from food fortification programs that there's any sort of increased risk that there's any sort of potential harm to fortification.

And that if you even look at intervention trials they've used like a really wide range of doses but some of it has gone up to five milligrams a day and there's no evidence of harm in relation to total or cancers or site specific cancers. I think that's a point of departure that generally additional folic acid in the food supply is safe for this particular outcome or the concerns that were expressed in relation to it as far as a reduction in risk or a potential benefit to folate in the food supply that that tends to in terms of and again this is where like you said cancer does get messy generally cuz it's an umbrella term for sites that really differ in kind of characteristics.

And otherwise from what I can see the most consistent kind of or the site specific cancer with the most research is in relation to colorectal cancer. Overall this does appear to in terms of observational research have a relationship with modestly lower risk of colorectal cancer overall. And this includes considering the introduction of food fortification as well specifically there's also suggestions, of a particular kind of temporal relationship between higher intakes of dietary folate and lower risk of cancer.

And this is a really important factor cuz with a lot of prospective cohort studies Cancers typically have quite long latency period as well. Again this is brought up statement cuz it depends on the site specific cancer but, if we're looking only at shorter term five year or maybe even 10 year periods there's the potential for some relationships to be missed.

And there was an analysis of UK data looking specifically at the post fortification period published last year from Walter Willet's group at Harvard. And I was looking at the nurses health study in particular but they looked specifically at a time course of folate equivalence looking at DFE.

And so per 400 micrograms of dietary folate equivalence there was a lower risk of colorectal cancer but that was. Dependent on the analysis relative to time of diagnosis. so for example where this was looked at in 16 to 20 years prior to diagnosis there was a 17% lower risk of colorectal cancer.

But that wasn't for example when it was only looked at over say 12 years or 12 to 16 years that was 7%. So suggestion of some sort of perhaps temporal relationship between a

higher dietary folate intake and potentially lower risk for colorectal cancer but certainly in the observational epidemiology that association overall appears to be one of the more.

Consistent in terms of direction of effect. and interestingly looking at you mentioned IBD earlier inflammatory bowel disease. I know that there's some evidence that if you're looking at colorectal cancer but specifically in a population with IBD which are at higher risk of these kinds of outcomes there's a more there's a greater effect size observed with folate supplementation in lowering risk of colorectal cancer in individuals with IBD. So ,that that appears to be the most kind of consistent association in terms of the epidemiology of folate and site specific cancer.

Danny Lennon: Maybe the last outcome that's worth looking at in in some degree of detail is relation to cognitive impairment. Alzheimer's disease. Maybe even specifically we've obviously talked about brain health and neurodegenerative disease in a number of previous episodes. And when you look at some of the dietary patterns that play a role, there obviously unsurprisingly things like high fruit and vegetable intake are associated with lower risk but that could be for many reasons one that has been put forward is these types of diets tend to be richer in folate.

And again this kind of gets linked back to elevated homocysteine in very much the same way or at least a mechanistic rationale that's discussed with cardiovascular disease. That tends to be one of the mechanisms involved here with cognitive impairment or neurodegenerative disease. ,maybe in in the same way we talked about with cardiovascular disease what do we actually know about first of all homocysteine in terms of risk of neurodegenerative disease how what kind of how strong that link is. And then after that then we can maybe consider folate more specifically.

Alan Flanagan: Again as a risk factor, whatever it is that homocysteine is representing as a risk factor either just a kind of a systems biomarker or causal biomarker, although as I said the evidence for a causal biomarker is not sufficient at this point, it is like cardiovascular disease is quite consistently and independently associated with risk of dementia. And Alzheimer's there's been a number of studies that have looked at this. And this what's interesting in the epidemiology is it's the independent often of B vitamin status or folate status in particular.

In the Framingham study for example there was and this was again at levels. So we mentioned earlier the trials of pretreatment baseline levels of 12 millimole per liter 12 micro per liter and. That this is the kind of threshold over which say 10 11 12 that you see quite substantial increases in risk for dementia Alzheimer's in the Framingham study it was a 40% higher risk over eight years. And these are in elderly participants at baseline. there was the Aberdeen birth cohort which looked at plasma homocysteine levels which were again plasma homocysteine levels were associated with lower folate and B 12 intakes but they weren't independently related to risk of dementia. Whereas the elevation in homocysteine was and ,that was a profound increase in dementia risk for dementia diagnosis.

In that cohort there was the Rotterdam MRI scan study where they it was took used MRI scans when a population based study. And what they showed in that study was that elevated homocysteine levels. Was associated with hippocampal atrophy this kind of brain region. That's really important for memory and cognition.

And so overall we've got these associations not just with homocysteine and the endpoint of dementia or Alzheimer's but associations with brain atrophy but in the epidemiology there it are inconsistencies then with B vitamins themselves. And there there are a lot of challenges again to with potentially trying to tease out B vitamins specifically in epidemiology. Particularly when you're like looking to report dietary intake where possibly for these actual biomarkers would be better indicators because again the high turnover ,and the high fluctuation rate in things like plasma but there are a number of interventions that have shed light and in many respects built on some of the homocysteine related observations and brain atrophy.

so the first was a trial the facet trial which was the folic acid and CIMT trial but they also looked at cognitive performance in a secondary analysis and were looking at and baseline. they had screened everyone for baseline homocysteine folate and B12. And what they showed was that supplemental folic acid resulted in yes significant reductions in homocysteine but a significant preservation of cognitive function over a three year period.

But that was a secondary endpoint. So it wasn't the main purpose of the trial but the actual potential role of this was more directly tested in the VITACOG study the homocysteine and B vitamins and cognitive impairment trial. And so what they looked at was a combination B vitamin supplement. So it was 0.8 milligrams or 800 micrograms of folic vitamin B 9.5 milligrams of B12 and 20 milligrams of vitamin B6.

And they assessed not just MRI brain atrophy rates but also cognitive testing. And the population was elderly adults with early mild cognitive impairment. and what they showed in this study was that B vitamin supplementation resulted in 30% less brain atrophy over two years compared to the placebo group.

And that effect was related to a nearly 23% reduction in homocysteine levels in that intervention group. And when they looked. Actual biomarker levels of folate and B12 the lower the or the rate of brain atrophy. This was related to greater levels of folate and B12. So as folate and B12 status went up the rate of brain atrophy slowed.

And this also in terms of magnitude of effect related to baseline homocysteine levels. So in participants with the highest baseline homocysteine levels again of over 11 12 milli per liter again that level threshold consistent with where profound exponential increase in risk of adverse cognitive outcomes and neurodegenerative disease is observed in the epidemiology.

In those with those highest baseline levels the rate of brain atrophy was 53% lower. So in fact the VITACOG trial was actually giving a lot of credence to more the kind of certainly homocysteine relationship. But the role of B vitamins and folate and B12 in this case in mediating rate of adverse cognitive outcomes and brain atrophy itself via reductions in homocysteine and the cognitive outcomes were also improved. But what this is really interesting is that the secondary analysis of the VITACOG study also came along and looked at the outcomes relative to baseline status of EPA and DHA. And what was interesting about that analysis was that the B vitamin supplementation had no effect in participants with low omega3 levels but the actual effect of B vitamin supplementation on protecting the brain against atrophy was highest.

In those with the highest baseline omega-3 fatty acid levels and that was stronger for DHA than it was for EPA. And so this interaction effect is something that has been noticed. And there's been a nice recent paper from Paul Fairburn and colleagues in Bournemouth where they looked at the effects of multi combined nutrient formulas of omega3 and B vitamins on cognition in older adults.

And it was published in the British journal of nutrition earlier this year. And what they found was actually the combination of omega3 and B vitamins leads to significant cognitive benefits in older adults when compared to placebos. So there does seem to be a potential interaction between them the 'whys and wherefores' of which have yet to be fully teased out but.

The the VITACOG trial and that recent systematic review and meta-analysis from Fairburn and colleagues certainly indicates that there's and this is a principle that again comes back to nutrition no nothing really exists in isolation. so there does appear to be an interactive effect between B vitamins and polyunsaturated fats DHA potentially in particular. BUT certainly the available intervention trial evidence to date is encouraging; of the of the beneficial effect of B nine and B12 in particular on not just cognitive testing but on what we might call hard endpoints like brain atrophy. and this effect does appear to be mediated by homocysteine levels such that the greater that homocysteine levels is reduced or in people who have very high baseline levels of homocysteine the magnitude of protection of the brain appears to be higher with supplemental B vitamins.

Danny Lennon: Yeah. And I think like you said this is fascinating not to consider just from this perspective but it raises a number of issues that happen across nutrition research more broadly. And I think people who heard the previous episode with a Kevin Klatt will have heard us discuss some issues that you and I have discussed in relation to omega three supplementation trials previously and how you can see why so much of that literature can be problematic when there's a failure to look at baseline DHA status achieve DHA status.

And then even now some of the picture around choline that Dr. Klatt highlighted is this is a consideration because we know something like choline can play a role in say DHA status here. Now we're seeing maybe potential interactive effects here between these omega

three fatty acids and something like folate. And again if these are interacting in some way to impact outcomes is something we need to know about and account for when we're appraising someone's literature. So that in itself is fascinating. But to that gives us a really good kind of overview of where we are in relation to this literature.

Before we finish I guess as is often the case when there are these. Complex areas where there are so many unknowns and so much more research needed in different areas. People can feel well what can we come away with right now? What are some conclusions we can come to? And then second like from a pragmatic practical perspective when it comes to getting adequate amounts of folate in the diet what does that typical advice end up being? So I wonder can we maybe touch on each of those for the moment? So first what do you think are the kind of you would condense down to what we can actually take from the folate and disease outcome literature right now or those kind of big takeaways to recap on. And then second we'll maybe discuss some pragmatic intakes after that.

Alan Flanagan: Yeah I think as far as a kind of hierarchy of needs in terms of evidence and application clearly for this particular nutrient consideration is for. women and couples planning a pregnancy indeed not every pregnancy is planned.

And this speaks to the importance of fortification. Something that you know to date a lot of European countries appear to be lagging behind on. But I would say that it's causal and unequivocal that folate deficiency or insufficiency results in increased risk for neural tube defects. and that supplementation periconceptually profoundly reduces risk of that adverse outcome.

And that that supplementation really should be in the kind of three months run up because this is an outcome that is very much related to early in the first trimester. And so it's repletion with folate prior to conception that ultimately is the factor that that reduces risk in this regard.

AND the evidence for that is is fairly overwhelming. as far as other related outcomes I don't know that we're at a point where one could say particularly from the cardiovascular disease perspective I think with the revolution that has occurred in recent years with lipid lowering therapy not only the kind of upgrading of statins with some of the more recent high intensity statins, the availability of PCSK9 inhibitors and the ability to treat to such a target level of the causal risk factor of LDL. ,it's difficult to say Hey there's a place for folic acid supplementation in here. certainly right now I think but again there that's not necessarily evidence against the importance of appropriate dietary intake and general folate repletion for that particular outcome as far as an overall picture of risk goes.

And then for the other outcomes we discussed like the neurodegenerative outcomes I think there's some real interest there. I think at this point it's difficult to make any sort of conclusive recommendations but I think if another couple of trials find. similar positive outcome then we could be getting to a point where you know it there is more kind of

direct ability to recommend B vitamin supplementation particularly in that specific population of older adults with some evidence of earlier mild cognitive impairment

Danny Lennon: In relation to just either people who want to make sure that their overall intake of folate is appropriate or then people maybe they're one of the people that do have one of these polymorphisms where maybe they're they require more folate intake to get to an appropriate status from a diet pattern overview based on what we've talked.

In terms of foods that typically contain folate what are what's the kind of big picture takeaway of what diets would allow someone to be fairly certain without having to go overly obsessive and tracking milligrams of intake that they can be relatively certain that their folate is a folate sufficient diet.

Alan Flanagan: Yeah ,the etymology of the word itself comes from the Latin for leaf. So that should hopefully be an indication dark green leafy veg; spinach lettuce broccoli et cetera all very rich in folate beans whole grains seafood eggs fruit. peanuts seeds so very possible to have a lot of folate intake kind of in independent of the type of diet and individuals following there's folate present in in foods of both animal and plant origin. So possible to get plenty of folate through the diet and indeed important to note that in countries that don't necessarily have mandatory fortification there are foods often that may be voluntarily fortified with additional folate. As far as some of the genetic factors. So I think we're called that we mentioned a study that had looked at 400 micrograms of dietary folate equivalents. And that could really be achieved with ,if we consider that a microgram of folic acid synthetic folic acid is about 1.7 micrograms of dietary folate equivalent. BUt either way if someone was to take 400 micrograms of folic acid it appears that independent of their MTHFR C 6 7 7 T genotype whether it's the heterozygous CT or the homozygous TT that will be sufficient to lower plasma homocysteine into normal ranges and to normalize blood folate red blood folate status. So yes people with that genetic variant would want to consider additional supplement. And for everyone else that there is the ability to obviously obtain. dietary folate through a fairly broad range of foods and food groups.

Danny Lennon: And for everyone listening if you are a Sigma Nutrition Premium subscriber there's gonna be pretty extensive study notes to this episode where I'll include not only like a glossary of terms but I'll put some of the relevant diagrams that help outline this folate and methylation cycle that we've discussed.

Some of the aspects of one carbon metabolism. That's just very much easier to grasp when you see that represented diagrammatically. And then also any of the trials and studies that we've mentioned throughout this conversation will be discussed with a quick overview of them and links to all that primary data as well and an explanation of anything else that we have covered. So you can check that out there. If you are currently not subscribed signature premium and you might be interested you can just look for the links somewhere in the description box but regardless. Alan and I will be back with another episode very soon. So I hope you've enjoyed this episode and I hope you join us for

another one in the near future. And until then I hope you have a great week and you stay safe and take care.