Detailed Study Notes: Episode 443

Kevin Klatt, PhD, RD – Choline

Episode Key Points

- Adequate intake of the omega-3 fatty acid DHA is advised during pregnancy, due to its role in brain and retinal development of the child, as well as decreased risk of preterm birth.
- Ingested DHA gets incorporated into different physiological pools, including the phosphatidylcholine-DHA pool.
- Additionally, pregnancy puts an increased stress on choline metabolism.
- Based on this, it has been hypothesized that choline demands may increase at this time, and that additional choline intake may increase DHA status.
- In a recently published RCT (1), researchers looked at the impact on DHA status in two groups:
    - Intervention
        - Choline 550 mg/d (500 mg + 50 mg tracer)
        - DHA 200 mg/d (algae)
        - Prenatal supplement
    - Control
        - Choline 25 mg/d (0 mg + 25 mg tracer)
        - DHA 200 mg/d (algae)
        - Prenatal supplement
- Results suggest that by supplementing choline alongside DHA (versus DHA alone), DHA bioavailability, and thus status, can be increased.
- This indicates that choline supplementation supports cellular metabolism to more efficiently handle and release DHA from a pregnant individual's liver, and ultimately be delivered into all tissues, including the placenta.

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Introduction to this Episode

A recently published study by Klatt and colleagues (1) examined the impact of choline supplementation alongside DHA supplementation, versus DHA supplementation alone, on DHA status in pregnancy.

It is known that DHA is a critical nutrient at this time for healthy development of the child. And through a number of mechanisms discussed later, it has been hypothesized that choline could lead to greater DHA status.

Terminology/Glossary

- **Methyl group:** A one-carbon (1C) unit.
- **One-carbon metabolism:** "One-carbon (1C) metabolism comprises a series of interlinking metabolic pathways that include the methionine and folate cycles that are central to cellular function, providing 1C units (methyl groups) for the synthesis of DNA, polyamines, amino acids, creatine, and phospholipids." (2)
- **Methyl donor:** Methyl donors are nutrients that will readily donate a methyl group to another substance. This occurs through the process known as the methylation cycle.
- **SAMe:** S-Adenosyl-L-Methionine is a chemical in the body made from methionine, an amino acid found in foods. It is a methyl donor (see above).
- **PEMT pathway:** phosphatidylethanolamine N-methyltransferase (PEMT) pathway
- **Isotope tracer:** An isotopic tracer is a radioactive atom that can be detected and therefore used to mark the compound we're examining, so that we can follow what happens to it. Tracer used in the discussed study was deuterium labeled-choline [d9-choline]. Tracers are explained more in the notes below.
- **RBC-DHA:** DHA found in the red blood cells.
- **PC-DHA:** DHA found in the membrane of phosphatidylcholine (PC).
- **TMAO:** Trimethylamine N-oxide (TMAO) is a choline metabolite that has been associated with a range of negative outcomes, mainly in relation to cardiovascular disease (you'll often hear it discussed in relation to debates about red meat for example). So theoretically there could be an issue with high cholines dosages, however this hasn't been shown to be problematic.

Connection to Previous Episodes

Episode 418

- Danny and Alan discussed the debate around whether a direct source of the long-chain omega-3 fatty acid DHA should be recommended.
- Based on the current state of the evidence, and the potential risks of DHA inadequacy, it was concluded that taking the 'precautionary principle' and getting a direct source of DHA in the peri-pregnancy period was important.
- For those who do not consume food sources of DHA (i.e. oily fish), then supplementation is advised.
- For those consuming a plant-exclusive diet, then this can be achieved with an algae-based supplement.

Episode 432

- Omega-3 fatty acid expert, Dr. Bill Harris was the guest in this episode.
- The role of DHA in brain development (as well as cognition in later life) was discussed at the 41:15 mark.
- Similar to episode 418, Dr. Harris' conclusion was that, while there are benefits to ALA (the omega-3 fatty acid in plants, that can be converted to EPA & DHA in the body), it is still better to get a direct source of preformed DHA (and EPA).

Episode 441

- Dietitian and maternal nutrition research Dr. Julie Abayomi was on the podcast discussing diet during pregnancy.
- The importance of DHA during pregnancy was discussed at the 25.50 mark.
- DHA is really important for brain and neurological development in the fetus.
- It's important to ensure adequate intake in early pregnancy
- There are studies that show that women who are deficient in omega-3 fatty acids in pregnancy have an increased risk of miscarriage and gestational diabetes.

What is Choline?

Humans can produce small amounts of the nutrient choline (via a pathway in the liver). However, this endogenously produced amount is usually inadequate to prevent deficiency, and so choline will need to be consumed through the diet (and/or supplementation).

Choline has several important functions, including being a part of the neurotransmitter acetylcholine and being a component of the major phospholipids in membranes (3).

Prolonged inadequate intake of choline may predispose individuals to nonalcoholic fatty liver disease and cognitive decline (3).

Dietary Sources

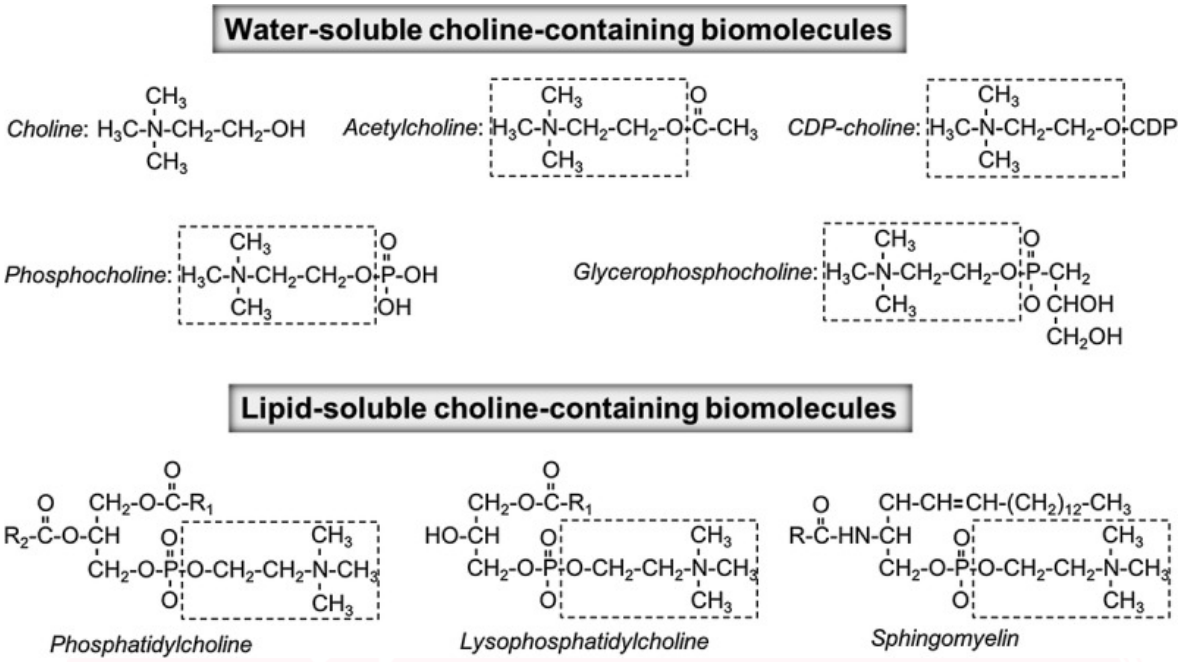
Choline is found in a range of foods, although animal products generally contain more choline per unit weight than plants.

Particularly good sources of choline (≥10% of the daily requirement per serving) include:

- Eggs (particularly yolks)
- Beef
- Chicken
- Fish
- Milk
- Cruciferous vegetables
- Certain beans

Lecithin is a mixture of fats that are essential to cells in the human body. For example, soy lecithin contains significant amounts of **phosphatidylcholine (PC)**, in addition to other compounds such as phosphatidylserine and phosphatidylinositol.

Choline can be found in many forms, which can be either water-soluble or fat-soluble. The different forms are also evident if you search for choline supplements, with several versions you have perhaps seen promoted. Some forms include: n-acetylcholine (NAC), phosphatidylcholine and alpha GPC (alpha glycerylphosphorylcholine)



Water-soluble and lipid-soluble choline-containing molecules found in food.
From: Wallace et al., Nutr Today. 2018 Nov-Dec; 53(6): 240–253 (4)

Foods also contain a metabolite of choline called betaine. Betaine can spare some choline requirements, it can be used as a methyl donor. Plant foods (particularly grains) can be a rich source of betaine. Many prepackaged foods add lecithin (i.e., phosphatidylcholine) and thus contribute to total dietary choline intakes.

Population	Age	AI, mg/d	UL, mg/d
Infants	0–6 mo	125 (18 mg/kg)	Not possible to establish[2]
	6–12 mo	150	Not possible to establish[2]
Children	1–3 y	200	1000
	4–8 y	250	1000
	9–13 y	375	2000
Males	14–18 y	550	3000
	≥19 y	550	3500
Females	14–18 y	400	3000
	≥19 y	425	3500
Pregnancy	All ages	450	Age-appropriate UL
Lactation	All ages	550	Age-appropriate UL

US Institute of Medicine's Adequate Intake (AI) and Tolerable Upper Limit (UL) for choline

Some estimates suggest only 11% of adults in the United States achieve the Adequate Intake (AI) for choline (5). In addition, some authors (including Dr. Klatt, (4)) have called for more dose-response studies and a re-visiting of appropriate intakes; specifically the need for the establishment of a Recommended Daily Allowance (RDA).

Currently there is no RDA established for choline due to lack of evidence, and therefore only an AI exists at present.

[Note: Recall the difference between an AI and RDA (6):

- ***Recommended Dietary Allowance (RDA)**: average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%-98%) healthy people.*
- ***Adequate Intake (AI)**: established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy.]*

	Mean Intake, mg/d[a]	AI[b], mg/d	Above AI, %[a]	UL[b], mg/d	Above UL, %
Males					
1–3 y	221 ± 7.6	200	61 ± 4.9	1000	0.0 ± 0.0
4–8 y	242 ± 4.2	250	42 ± 2.5	1000	0.0 ± 0.0
9–13 y	290 ± 5.2	375	14 ± 1.8	2000	0.0 ± 0.0
14–18 y	346 ± 11.1	550	5 ± 2.1	3000	0.0 ± 0.0
19–30 y	412 ± 7.5	550	17 ± 2.2	3500	0.0 ± 0.0
31–50 y	421 ± 9.1	550	14 ± 2.5	3500	0.0 ± 0.0
51–70 y	391 ± 7.8	550	10 ± 1.6	3500	0.0 ± 0.0
≥71 y	351 ± 8.2	550	4 ± 1.1	3500	0.0 ± 0.0
Females					
1–3 y	205 ± 4.8	200	40 ± 4.1	1000	0.0 ± 0.0
4–8 y	211 ± 4.7	250	20 ± 3.3	1000	0.0 ± 0.0
9–13 y	231 ± 7.2	375	<3	2000	0.0 ± 0.0
14–18 y	223 ± 7.9	400	<3	3000	0.0 ± 0.0
19–30 y	271 ± 6.6	425	<3	3500	0.0 ± 0.0
31–50 y	280 ± 5.5	425	5 ± 1.5	3500	0.0 ± 0.0
51–70 y	275 ± 5.4	425	4 ± 1.0	3500	0.0 ± 0.0
≥71 y	253 ± 6.0	425	<3	3500	0.0 ± 0.0

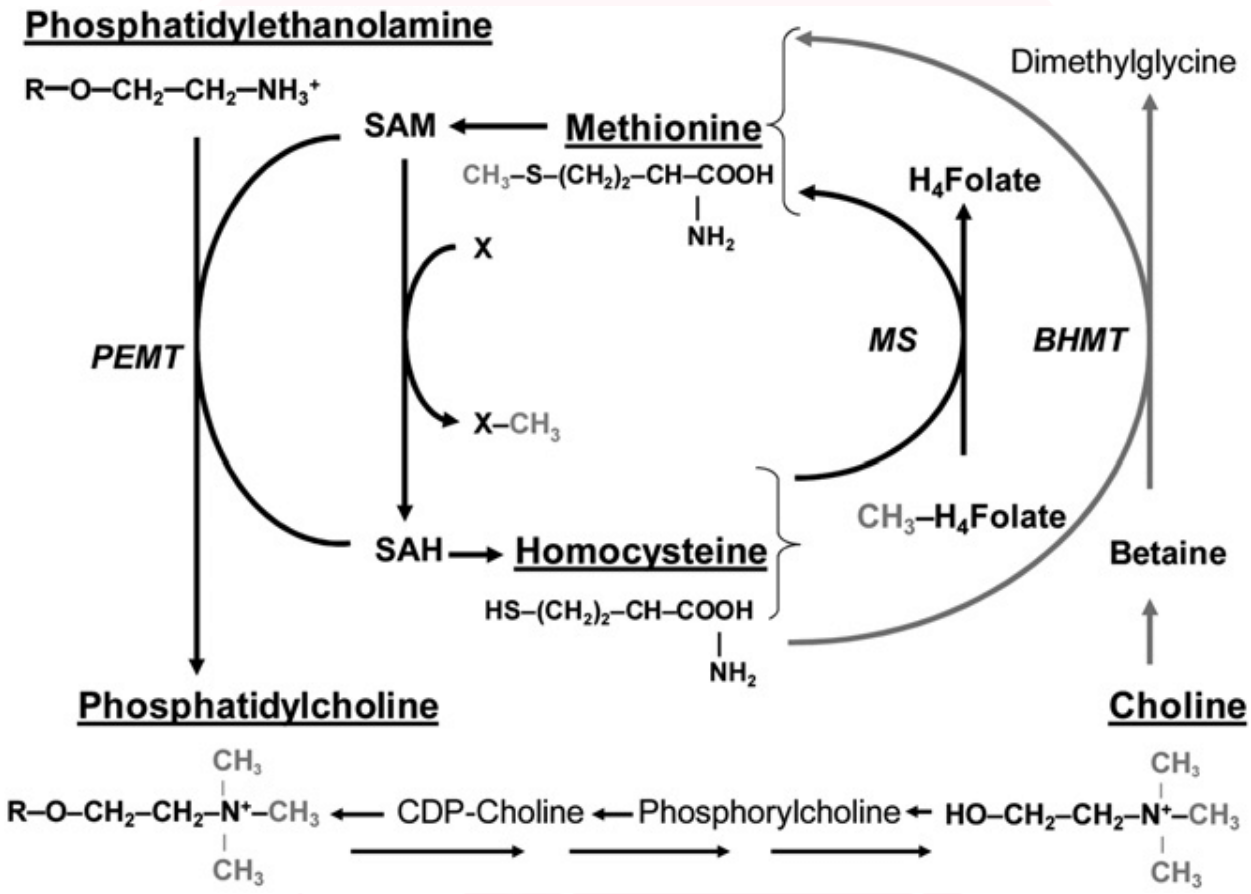
Abbreviations: AI, adequate intake; UL, upper intake limit.
[a]Data source: What We Eat in America, National Health and Nutrition Examination Survey (NHANES) 2011–2014, individuals 1 year or older, excluding breast-fed children and pregnant or lactating women. Provided courtesy of Alanna Moshfegh.[8]
[b]Current gender and life-stage AI as defined by the National Academy of Medicine Food and Nutrition Board.[1]

Table taken from: Wallace *et al*., Nutr Today. 2018 Nov-Dec; 53(6): 240–253 (4)

One-Carbon Metabolism Basics

One-carbon metabolism: *"One-carbon (1C) metabolism comprises a series of interlinking metabolic pathways that include the methionine and folate cycles that are central to cellular function, providing 1C units (methyl groups) for the synthesis of DNA, polyamines, amino acids, creatine, and phospholipids."* (2)

Methyl donor: Methyl donors are nutrients that will readily donate a methyl group to another substance. This occurs through the process known as the methylation cycle.



Interaction of folate and choline, and the methylation processes.
From: Troen et al., 2009 - Journal of Nutrition 138(12):2502-9 (7)

DHA & Pregnancy

- The infant "brain growth spurt" occurs during the last trimester through the first 2 years of infancy (8).
- During pregnancy DHA accumulates in fetal tissues; getting transferred from the maternal compartment by the placenta. DHA is then incorporated into fetal nervous system cell membranes.
- "DHA accretion in the brain accelerates during the middle of gestation, slows down in infancy, and reaches a plateau in early adulthood" (9).
- This accretion of DHA in fetal tissues is thought to be supported by around 200 mg/d of DHA.
- Maternal DHA levels are dependent on dietary intake and maternal DHA status appears to be the strongest predictor of cognitive development.
- DHA adequacy is linked to brain/retinal development & decreased risk of preterm birth.
- Supplementation of DHA during pregnancy is often advised, either:
    - 200 mg/d in addition to 1-2 servings of low-mercury oily fish
    - 600 mg/d, if there is no food-based intake of DHA

Previous work on choline suggested that high maternal choline intake eases an infant's response to stress, improves information processing and has long-term benefits in sustained attention (10).

DHA Pools in the Body – Relevance of Choline

Ingested DHA gets incorporated into different physiological pools. However, there's still debate over which pools contribute the most to the accretion of DHA in fetal tissues.

DHA makes up over 90% of omega-3 in the brain and 10%–20% of the brain's total lipids. DHA is stored in the membrane phospholipids of (9):

- phosphatidylethanolamine (PE)
- phosphatidylserine (PS)
- **phosphatidylcholine (PC)**

After oral ingestion of DHA, unesterified DHA free fatty acid (DHA-FFA) are delivered to the small intestine. These are processed by the small intestine and the liver, and the DHA is converted into several different versions that can then go into circulation, namely:

- DHA-triacylglycerides (DHA-TAGs)
- DHA-PC
- DHA-FFA bound to low density lipoprotein (LDL) and albumin

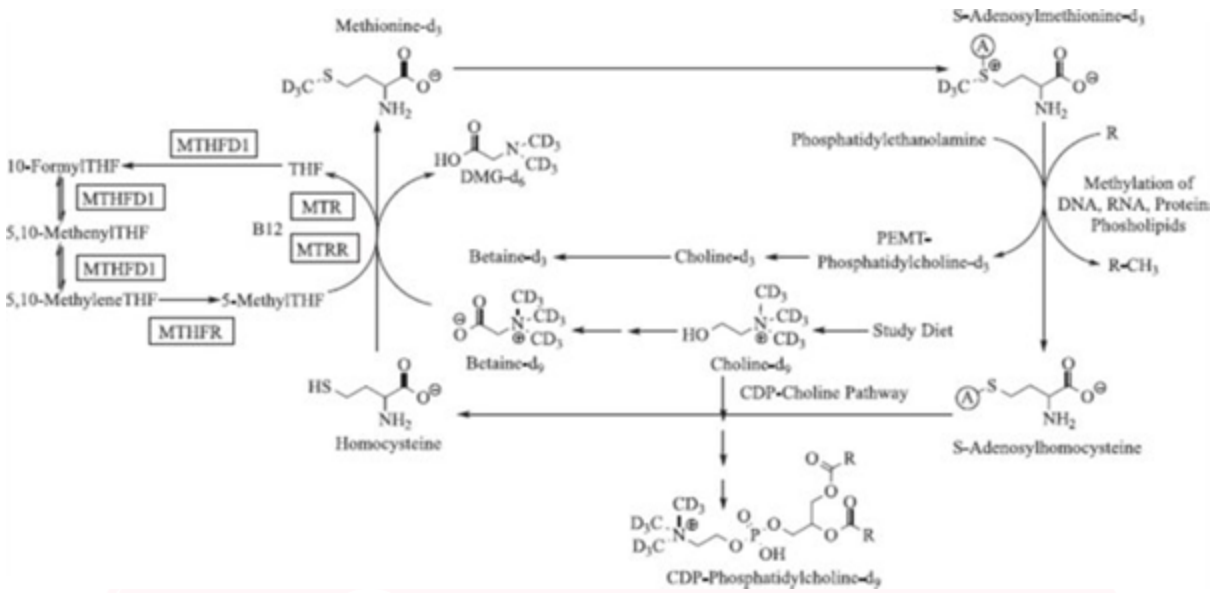
The focus of the study discussed in the episode was on the PC-DHA pool. This specific pool is of relevance to pregnancy for several reasons:

1. PC-DHA is mostly produced in the maternal liver, via the activity of an enzyme called **phosphatidylethanolamine N-methyltransferase (PEMT).**
2. PEMT activity is influenced by estrogen. So higher levels of estrogen lead to increased PEMT activity.
3. Estrogen rises substantially across pregnancy, with maximal concentrations occurring in the 3rd trimester. These can get up to 10x normal levels. (Recall that the 3rd trimester is also the period when there is significant DHA accretion in the fetal brain tissue).
4. PEMT utilizes methyl groups. Methyl groups (which include those that are derived from choline) seem to be limited to some extent in pregnancy.

In addition, choline metabolism is strained during pregnancy. Mice studies show that there is likely mobilization of choline from the maternal liver to the fetus (11,12).

The hypothesis that supplementing choline alongside DHA (versus DHA alone) would improve the response to DHA (and therefore improve DHA status) is built on the basis that:

1. DHA is important in pregnancy.
2. During pregnancy, estrogen increases substantially, peaking in the 3rd trimester at potentially 10-fold the normal levels.
3. Increased estrogen likely increases PEMT activity.
4. Choline metabolism is also stressed at this time. This would limit the response to DHA intake.
5. Therefore increasing choline intake could lead to more of the DHA being available to be delivered to fetal tissues.



Klatt et al., 2022 – Study Design

The study of focus in this episode (1) was a RCT with 2 groups:

1. **Intervention**: Choline 550 mg/d (500 mg + 50 mg tracer) + DHA 200 mg/d (algae) + prenatal supplement
2. **Control**: Choline 25 mg/d (0 mg + 25 mg tracer) + DHA 200 mg/d (algae) + prenatal supplement

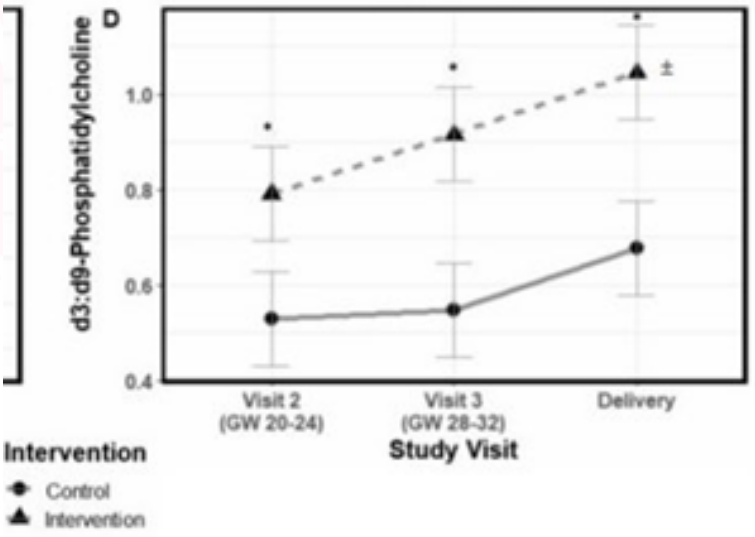
Control	Intervention
Choline 0mg/d *25 mg/d d9-choline*	Choline 500mg/d *Choline chloride (Balchem Inc)* *50 mg/d d9-choline*
DHA 200 mg/d *From Algal microalgae oil; DSM*	DHA 200 mg/d *From Algal microalgae oil; DSM*
Prenatal/d Nature Made Prenatal Tablet	Prenatal/d Nature Made Prenatal Tablet

This dose of choline in the intervention group (550 mg) has been previously linked to cognitive benefits & improved concentrations of methyl donor metabolites. It also goes slightly beyond the AI for pregnancy, and is above the average choline intake in the population. However, it is important to note that this dose is supplemental choline (not total dietary choline). More on this later.

What is a “tracer”?

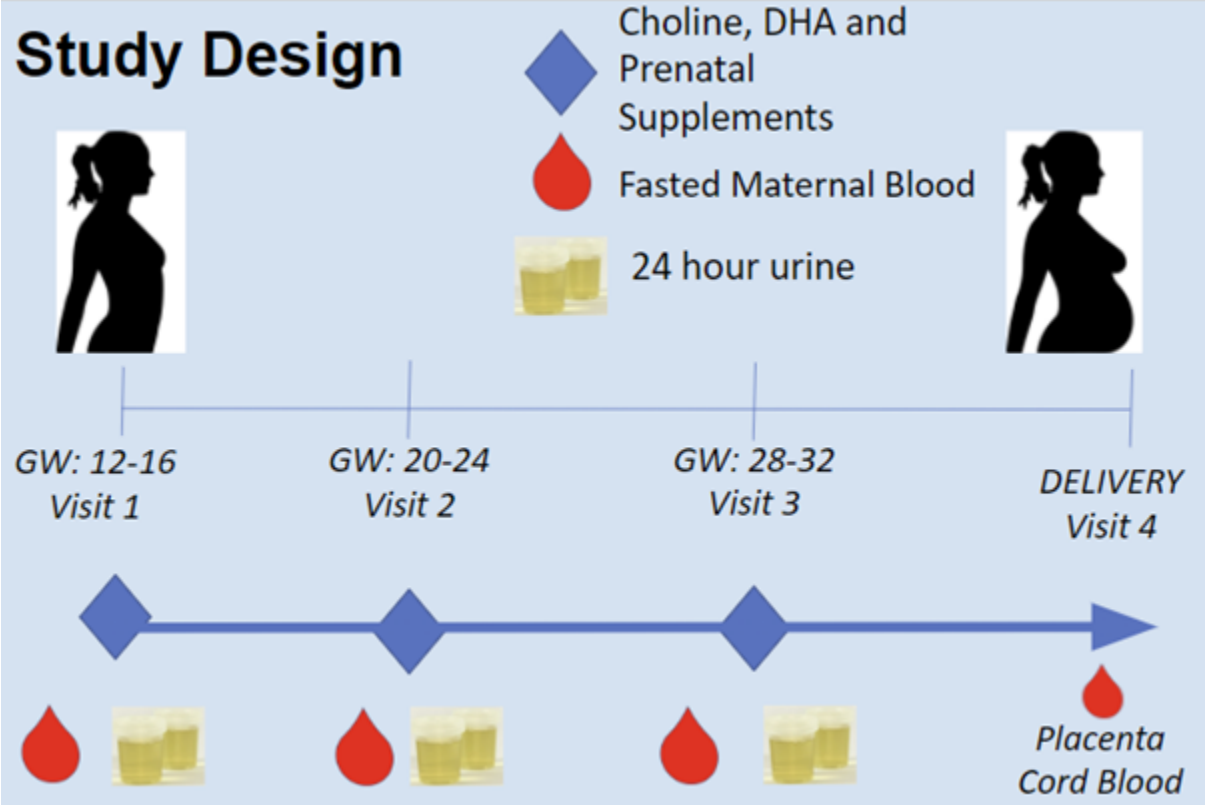
- Using tracers is essentially a method that allows us to “trace” what happens to a compound in the body. In other words, it allows us to see how it’s distributed and its ultimate fate.
- A tracer is more specifically an isotope tracer (or isotopic tracer).
- An isotopic tracer is a radioactive atom that can be detected and therefore used to mark the compound we’re examining, so that we can follow what happens to it.
- Of course, an isotopic tracer must not behave differently to the regular compound that is not marked with a tracer. The tracer cannot alter its action. But at the same time, it has to have some distinguishing feature that can be detected as different to “normal material” so that we can see where it ends up.
- As an example, we can see if a fatty acid ends up in certain cell membranes by using a tracer study to see if the tracer-labeled fatty acids are detected in the cell membrane.

In this study, the researchers used a “methyl-d9-choline tracer” to estimate PEMT activity. They looked at plasma levels of phosphatidylcholine (PC) and its d9 and d3 (indicator of PEMT activity) enrichments. The researchers stated that this data suggested that there is a significant increase in PEMT activity.



Participants entered the study at between 12-16 weeks of the pregnancy. There were then follow-up visits at 20-24 weeks, 28-32 weeks, and then at delivery.

At each visit fasted blood and 24-hour urine was collected. And at delivery placenta cord blood was also collected.



Overview of Results

- The pattern of DHA metabolite response in plasma and red blood cells (RBCs) was consistent with the researchers' hypothesis.
- The most statistically significant & largest in magnitude effects were apparent for the validated DHA status marker, RBC total DHA.
- The listed primary outcome was RBC PC-DHA, which did not meet statistical significance of $P<0.05$ (it was $P=0.11$). However, the better marker to look at is likely RBC-DHA…
- Dr. Klatt mentioned that at the delivery time-point collection, some of the data collected was a bit inconsistent, perhaps due to changes that occur acutely at delivery. This threw off some models. Therefore, looking at the most robust and well validated marker of DHA status, RBC-DHA, is best.

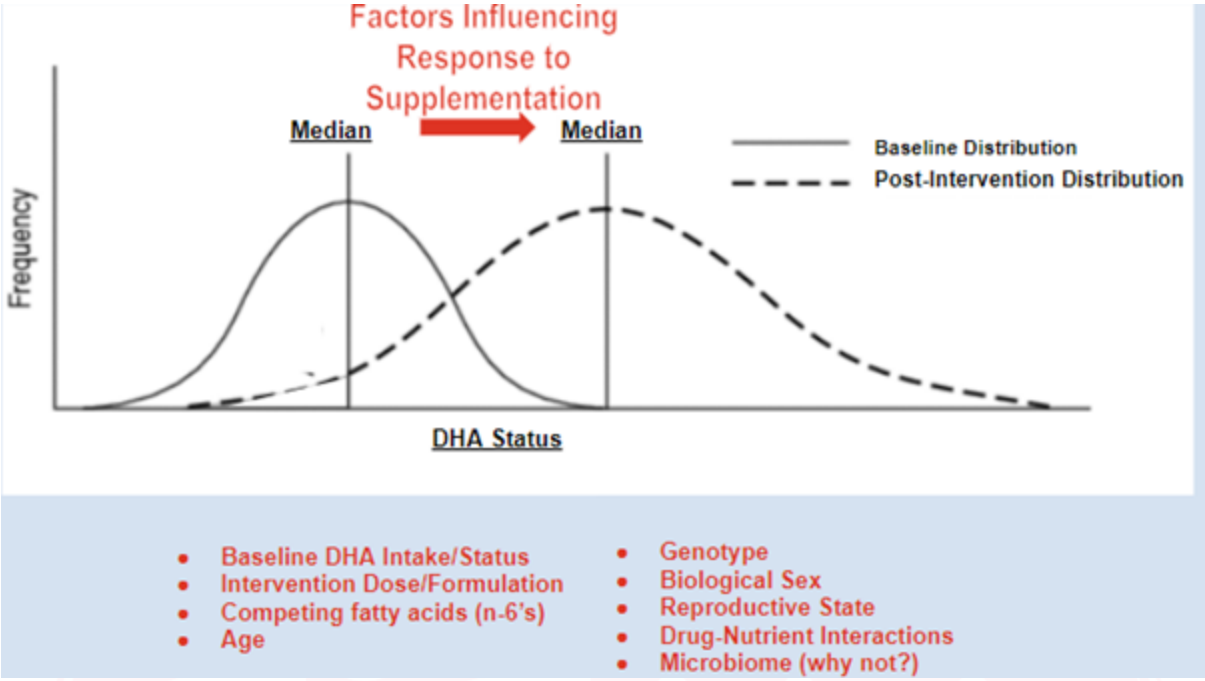
Conclusions

- The findings show that choline supplementation supports cellular metabolism to more efficiently handle and release the DHA from a pregnant individual's liver into the bloodstream, and then ultimately delivered into various tissues, including the placenta.
- Therefore by supplementing choline and DHA together (as opposed to DHA alone), it is possible to increase DHA bioavailability and hence improve DHA status beyond what would be achieved without choline.
- This highlights something that has actually been known by researchers for quite a while; that dietary DHA intake is only a partial determinant of DHA status gains. In pregnancy, it's very likely that DHA status is also driven by methyl metabolism (dietary adequacy, genetic variants, etc), of which choline is an important part.

Related Point: Nutrition Trials & DHA Data

In the podcast episode, Dr. Klatt mentioned some issues that this highlights with respect to both nutrition trials broadly, and then the data on DHA up to this point specifically. Some things Dr. Klatt noted:

1. Nutrition, especially for omega-3 trials, is tough to study with randomized trials.
2. Often, we are targeting improving DHA status, without using study designs that take into account baseline status & other factors that modify the response to supplementation. This leads to more "noise" in the data than we'd like; making it hard to detect the "signal".
3. If we don't know baseline status (and other factors) then we're not isolating the impact of the nutrient in question (see image below).
4. With respect to the DHA supplementation literature, Dr. Klatt stated that it's very likely that the response to DHA supplementation has been limited by intervening in choline-stressed pregnant populations.
5. As a comparative analogy, Dr. Klatt mentioned the example of studying the impact of calcium supplementation in a lower vitamin D status population, without knowing their vitamin D status or even connecting it to calcium (Without enough vitamin D there is insufficient calcium absorption from the diet).



Practical Application

Some potential take-aways for practice:

1. Currently there are no set guidelines for choline supplementation at pregnancy.
2. However, in cases where improving DHA status is the goal, this evidence gives a good basis to believe choline would be of benefit.
3. Dr. Klatt noted however that it's challenging to relate choline supplementation (like used in this study as a water-soluble salt) to food-derived choline (which is mainly in the form of fat-soluble PC). Klatt stated that he believes it is highly questionable whether these will have the same metabolic effects.
4. Choline has other beneficial effects other than simply improving DHA status. And so these are other reasons to see it as an important nutrient.
5. In lieu of more evidence and guidelines, as of right now it’s up to individual health professionals to make decisions based on what evidence is available. It seems that the dosages of choline supplementation used in this study (~ 500 mg/d) are safe and unlikely to cause issues.

Danny's Key Ideas

The main things to take from this discussion, in my view, are:

1. Adequate intake of the omega-3 fatty acid DHA is advised during pregnancy, due to its role in brain and retinal development of the child, as well as decreased risk of preterm birth.
2. Ingested DHA gets incorporated into different physiological pools, including the phosphatidylcholine-DHA pool.
3. Additionally, pregnancy puts an increased stress on choline metabolism.
4. Based on this, it has been hypothesized that choline demands may increase at this time, and that additional choline intake may increase DHA status.
5. In a recently published RCT (Klatt et al. 2022), researchers looked at the impact on DHA status in two groups:
    a. Intervention
        i. Choline 550 mg/d (500 mg + 50 mg tracer)
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6. Results suggest that by supplementing choline alongside DHA (versus DHA alone), DHA bioavailability, and thus status, can be increased.
7. This indicates that choline supplementation supports cellular metabolism to more efficiently handle and release DHA from a pregnant individual's liver, and ultimately be delivered into all tissues, including the placenta.

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