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Making Animals Smarter: Nutrition & Cognition

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Strategic Development Manager, Nutrition Science

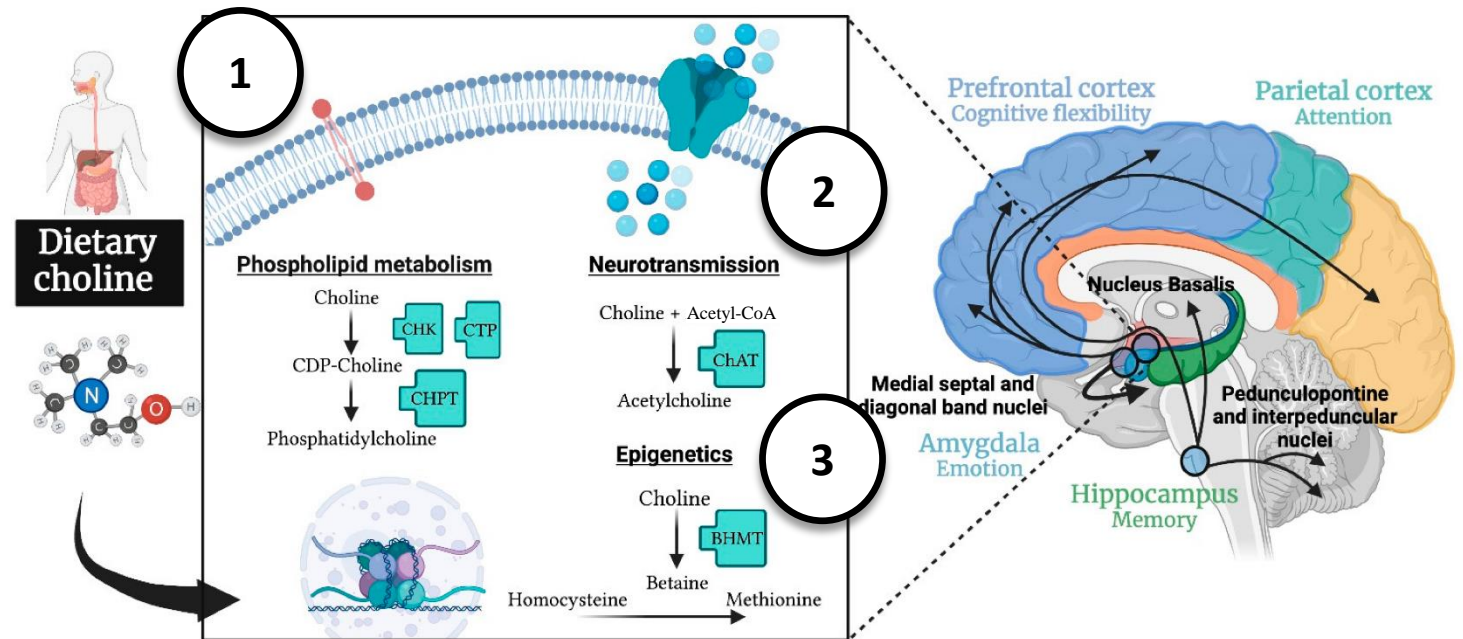
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Choline Supports Multiple Aspects of Brain Health



Choline has multiple roles in brain health:

- 1) **Maintains cell membrane integrity** by regulating phospholipid metabolism
- 2) **Helps support nervous system function** by acting as a component of the neurotransmitter Acetylcholine
- 3) **Helps regulate gene expression** in key brain areas, which **supports mood, memory, and attention**



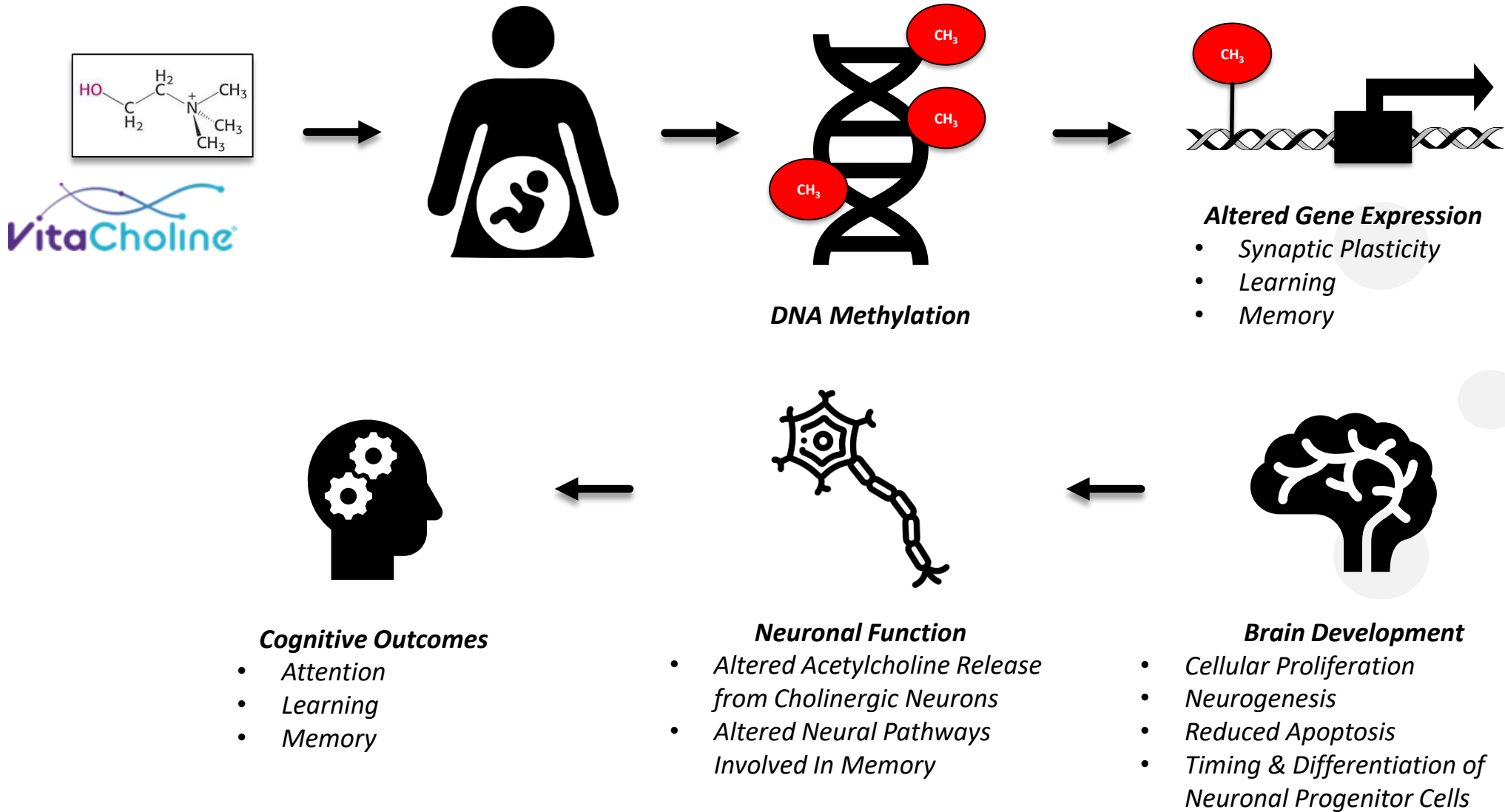
Gamiz F & Gallo M; *Nutrients* 2021; 13(6):1966; doi: 10.3390/nu13061966.

Animal Studies Connect Maternal Choline Intake and Offspring Cognition

Citation	Animal	Choline Intervention	Cognition Assessment	Offspring Age at Assessment	Results
Meck WH et al., <i>Dev Psychobiol</i> 1988; 21: 339-353.	Pregnant Rats	2d prior to conception through birth	<ul style="list-style-type: none"> 12 & 18 arm radial arm maze 	60 days	<ul style="list-style-type: none"> ↑ Visuospatial Memory
Glenn MJ et al., <i>Brain Res</i> 2008; 1237: 110-123.	Pregnant Rats	E12-E17	<ul style="list-style-type: none"> Open field exploration Novel object exploration Hippocampal Plasticity (IHC, Stereology) 	1 month 24 months	<ul style="list-style-type: none"> Associated w/exploratory behavior ↑ Hippocampal plasticity
Meck WH, Williams CL; <i>Brain Res</i> 1999; 118: 51-59.	Pregnant Rats	E11-E18	<ul style="list-style-type: none"> 12 arm radial arm maze 	120 days	<ul style="list-style-type: none"> ↑ Accuracy
Meck WH, Williams CL; <i>Neuroreport</i> 1997; 8: 3053-3059.	Pregnant Rats	E12-E17	<ul style="list-style-type: none"> 6, 12, 18, & 24 radial arm mazes 	60 days	<ul style="list-style-type: none"> ↑ Spatial memory (“chunking”)
Meck WH, Williams CL; <i>Neuroreport</i> 1997; 8: 3045-3051.	Pregnant Rats	E11-E18	<ul style="list-style-type: none"> Peak interval timing procedure 	4-6 months 24-26 months	<ul style="list-style-type: none"> Suppl. ↑ Cognitive Function Deficiency ↓ Attention

Adapted From: Irvine N, et al., *Nutrients* 2022; 14(2): 364.

How Does Choline Impact The Developing Brain?



Adapted From: Irvine N, et al., *Nutrients* 2022; 14(2): 364.

Choline Intake During Pregnancy Is Associated With Childhood Cognition

Study Goal

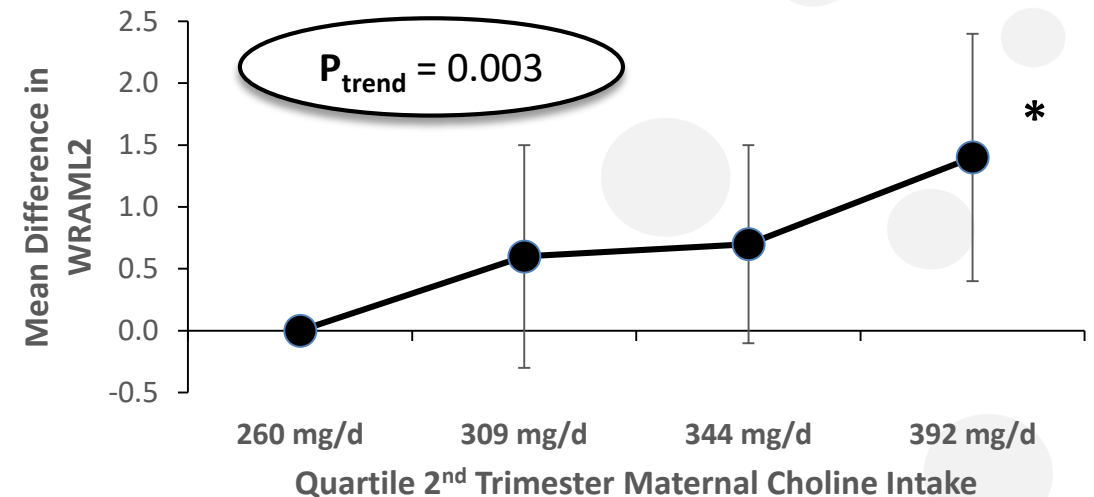
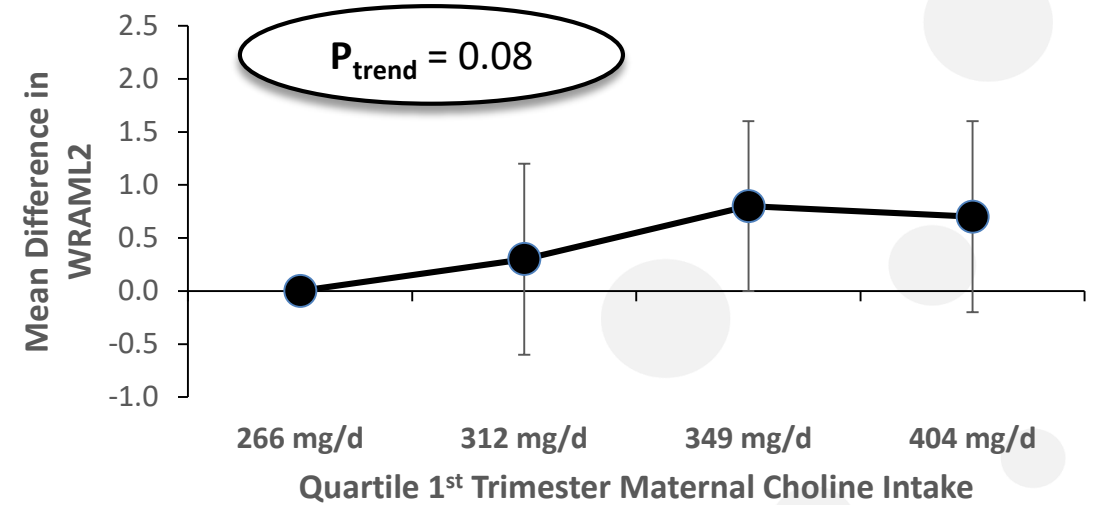
To examine maternal 1st and 2nd trimester dietary intake of methyl donor nutrients during pregnancy in relation to child visual memory

Study Design

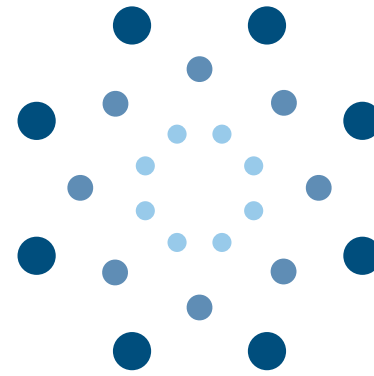
- **Design:** Prospective Cohort Study
 - N=895 mother/child pairs
- **Outcomes:**
 - Intake of methyl donor nutrients during 1st and 2nd trimesters of pregnancy
 - Offspring Visual Memory
 - Wide Range Assessment of Memory and Learning, 2nd edition (WRAML2)

Results & Conclusions

Higher gestational choline intake was associated with better child visual memory at age 7



Boeke CE, et al., Am J Epidemiol 2013; 177(12): 1338-1347;



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Low availability of choline *in utero* disrupts development and function of the retina

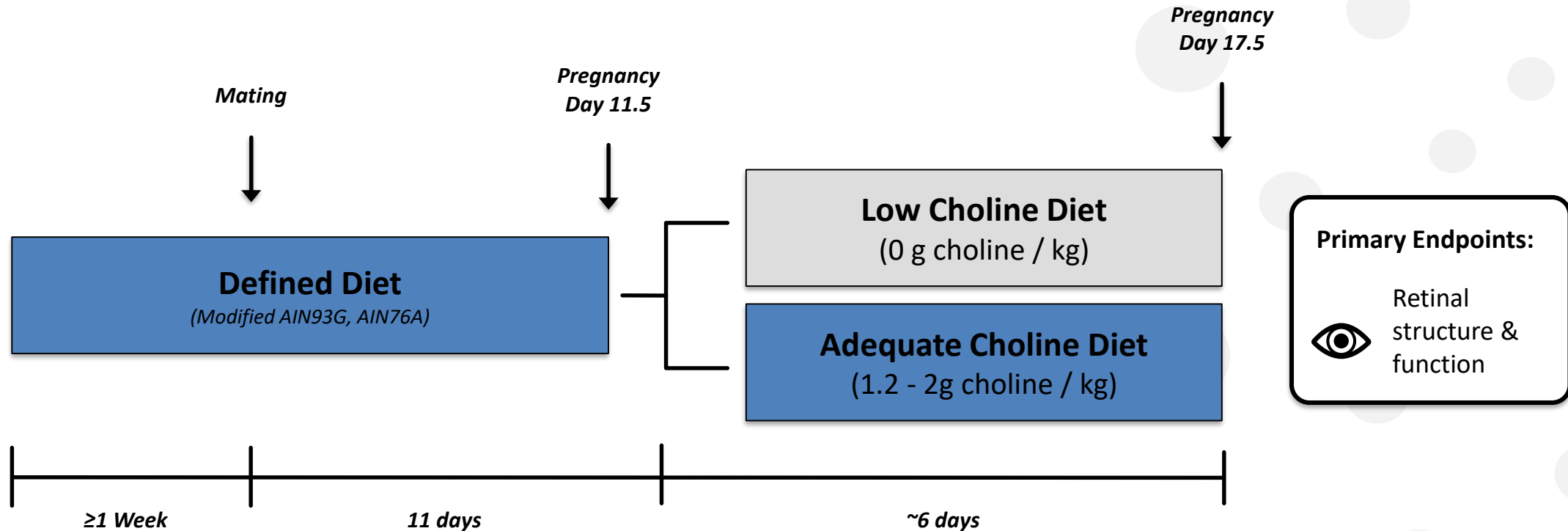
Trujillo-Gonzalez I, et al., *FASEB J* 2019; 33: 9194-9209.

How Does Maternal Choline Intake Impact Eye Development?

Study Goal: To address the role of *in utero* choline supply for the development and function of the retina in a mouse model



Female C57BL/6J mice
Age 35d-4 months

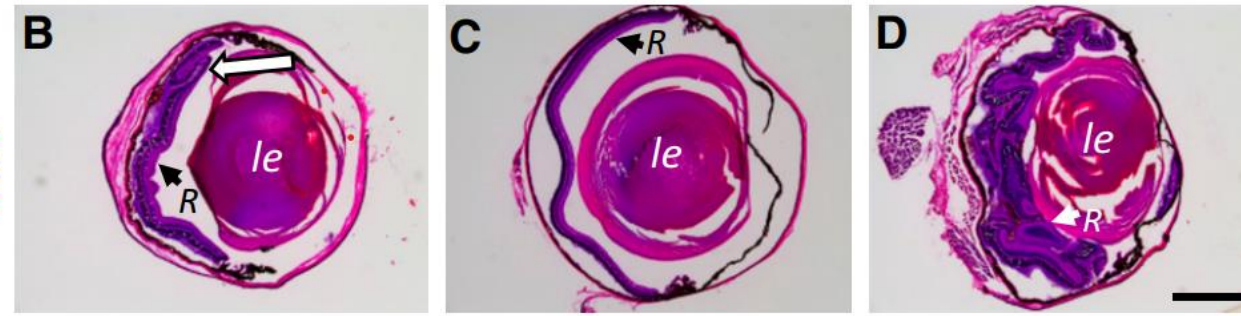
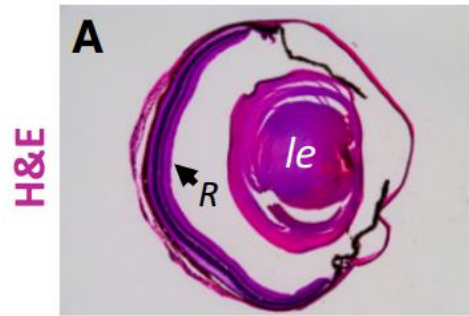


Trujillo-Gonzalez I, et al. *FASEB J* 2019; 33: 9194-9209.

How Does Maternal Choline Intake Impact Eye Development?

Adequate choline

Low choline

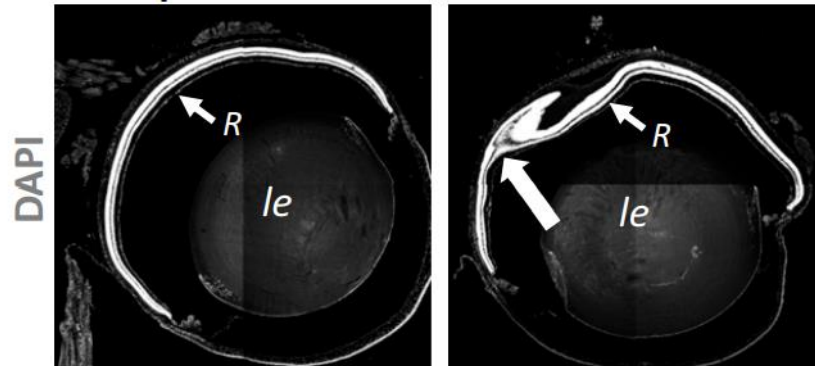


E11.5 → E17.5

E11.5 → P3

E Adequate choline

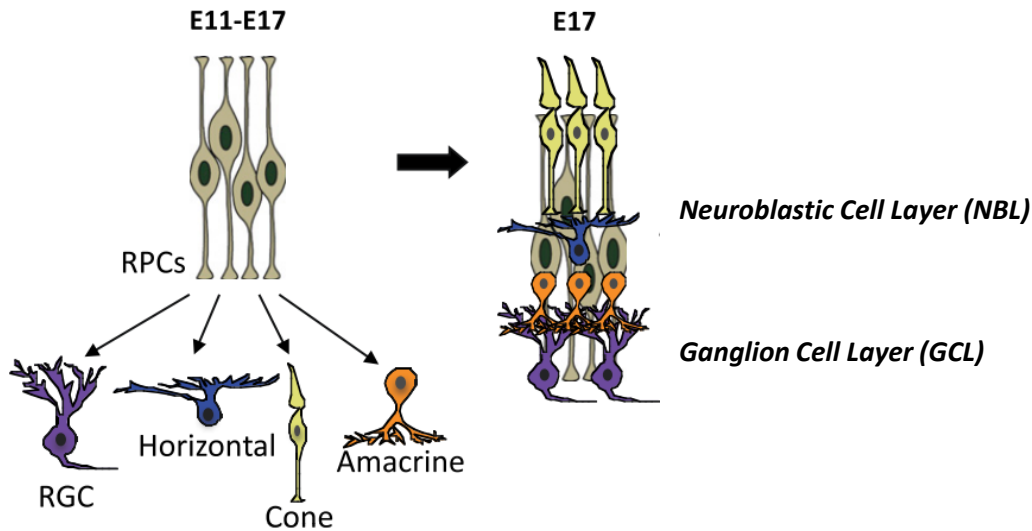
Low choline



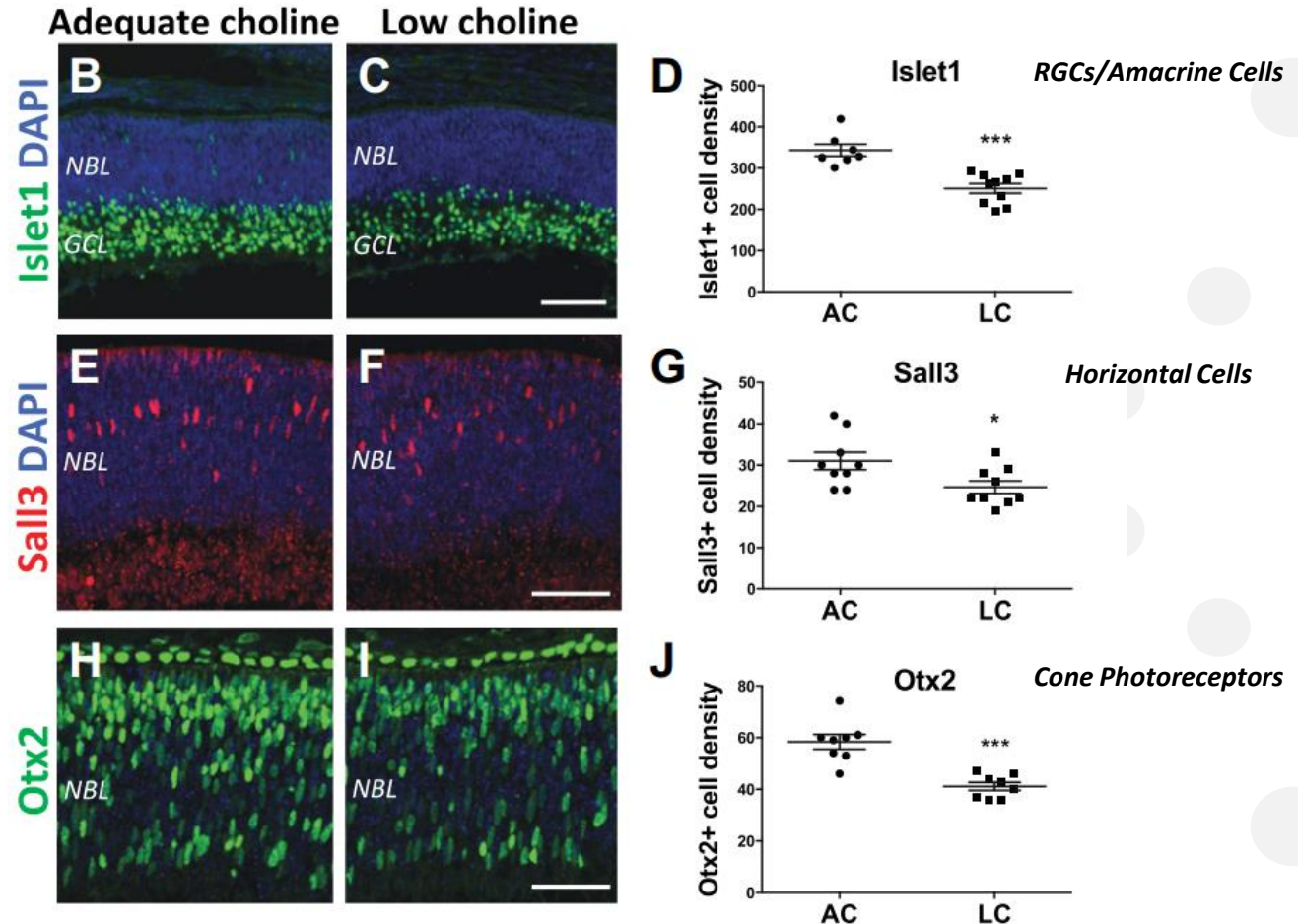
- **Low choline during pregnancy results in abnormal retinal development in the offspring such as retinal folding & hypocellularity**
- **Choline supports normal eye development during pregnancy**

Trujillo-Gonzalez I, et al. *FASEB J* 2019; 33: 9194-9209; le = left eye; R = retina; H&E = Hematoxylin & Eosin;

How Does Maternal Choline Intake Impact Eye Development?

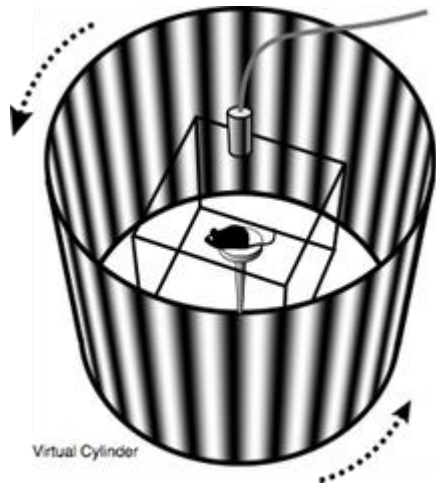


- **Retinal Progenitor Cells (RPCs)** differentiate into several cell types during normal eye development
- **RPC differentiation is decreased when dietary choline is limited**

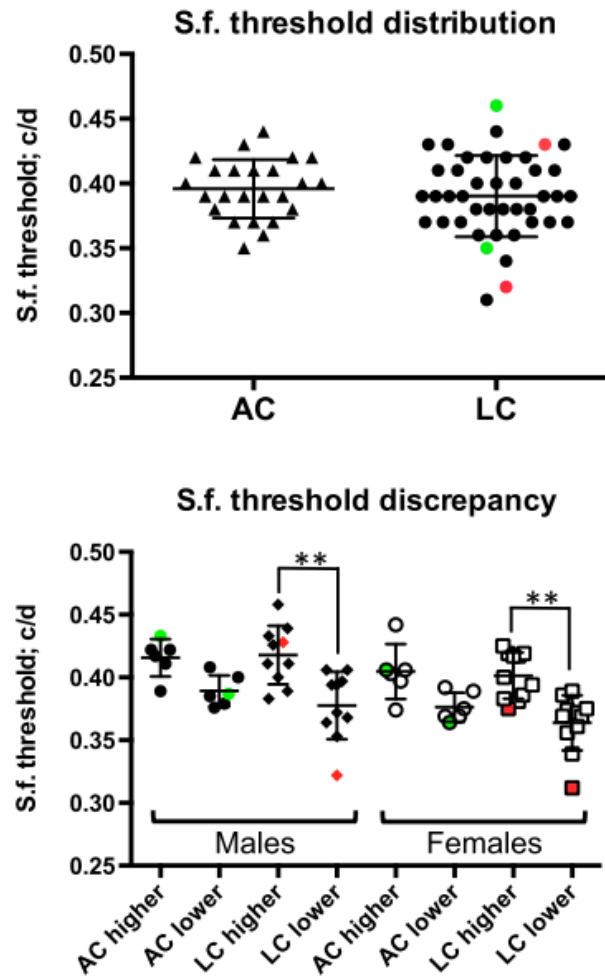


Trujillo-Gonzalez I, et al. *FASEB J* 2019; 33: 9194-9209;

How Does Maternal Choline Intake Impact Eye Function?



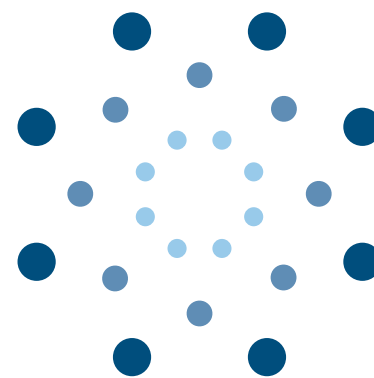
Optokinetic Response: Allows the eye to follow objects in motion when the head remains stationary



- **On average**, optokinetic motor tracking thresholds in the pups was not impacted by maternal choline intake
- Pups born to low choline consuming mothers showed differences in **visual sensitivity between the left and the right eyes**

Choline supports the normal development of eye function

Trujillo-Gonzalez I, et al. *FASEB J* 2019; 33: 9194-9209; <http://www.cerebralmechanics.com/how-optometry-works/>; sf = spatial frequency



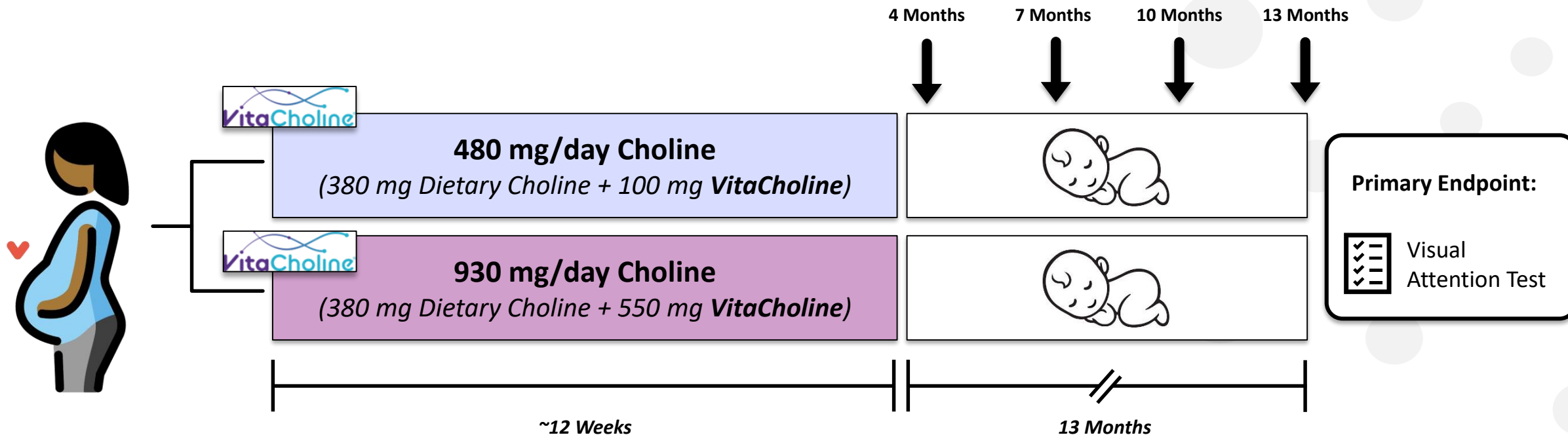
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**Maternal choline supplementation during the third trimester of pregnancy improves infant information processing speed:
A randomized, double-blind, controlled feeding study**

Caudill MA, et al., *FASEB J* 2018; 32(4): 2172-2180

Does Maternal Choline Supplementation Improve Baby's Brain Health?

Study Goal: To examine the effects of maternal choline supplementation during pregnancy on infant cognition



N=26 pregnant women,
entering 3rd trimester;
age ≥ 21y;

Caudill MA, et al., *FASEB J* 2018; 32(4): 2172-2180; All subjects also received OTC prenatal multivitamin, 200 mg DHA, supplemental K & Mg; Choline supplied as Choline Chloride

How Do You Measure Cognition in Infants?

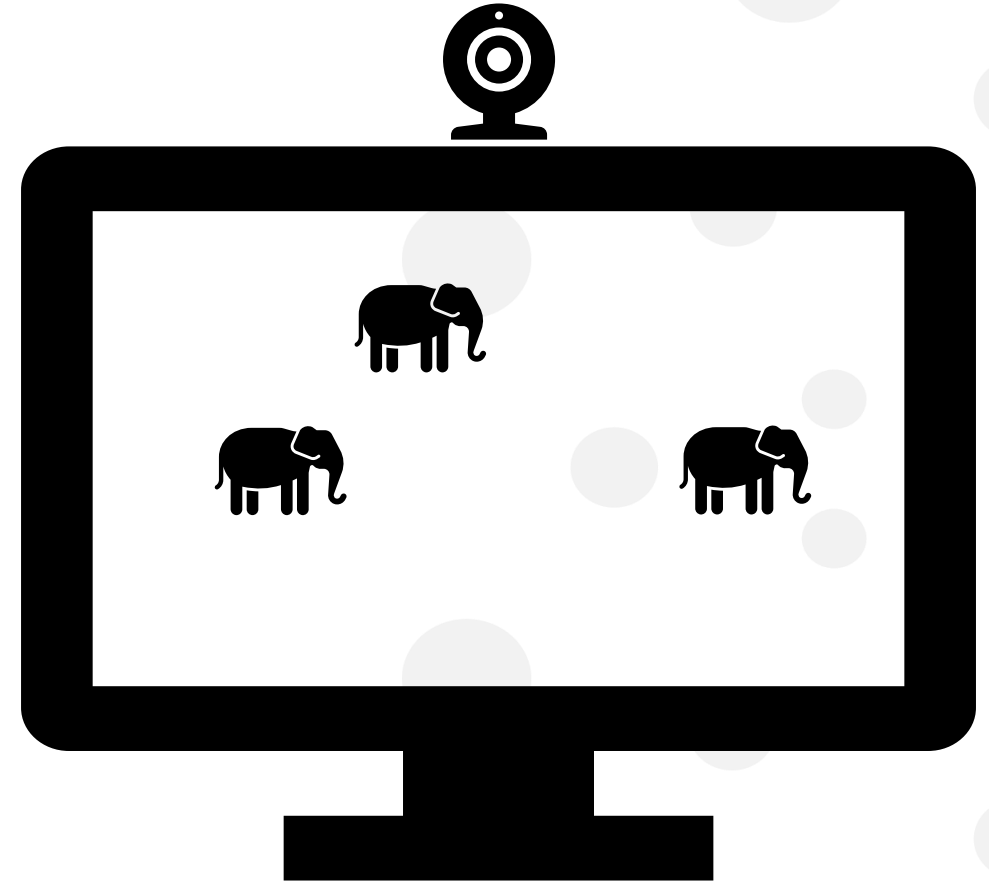
Primary Endpoint:

Visually Guided Reactive Saccades

Measures: Information Processing Speed

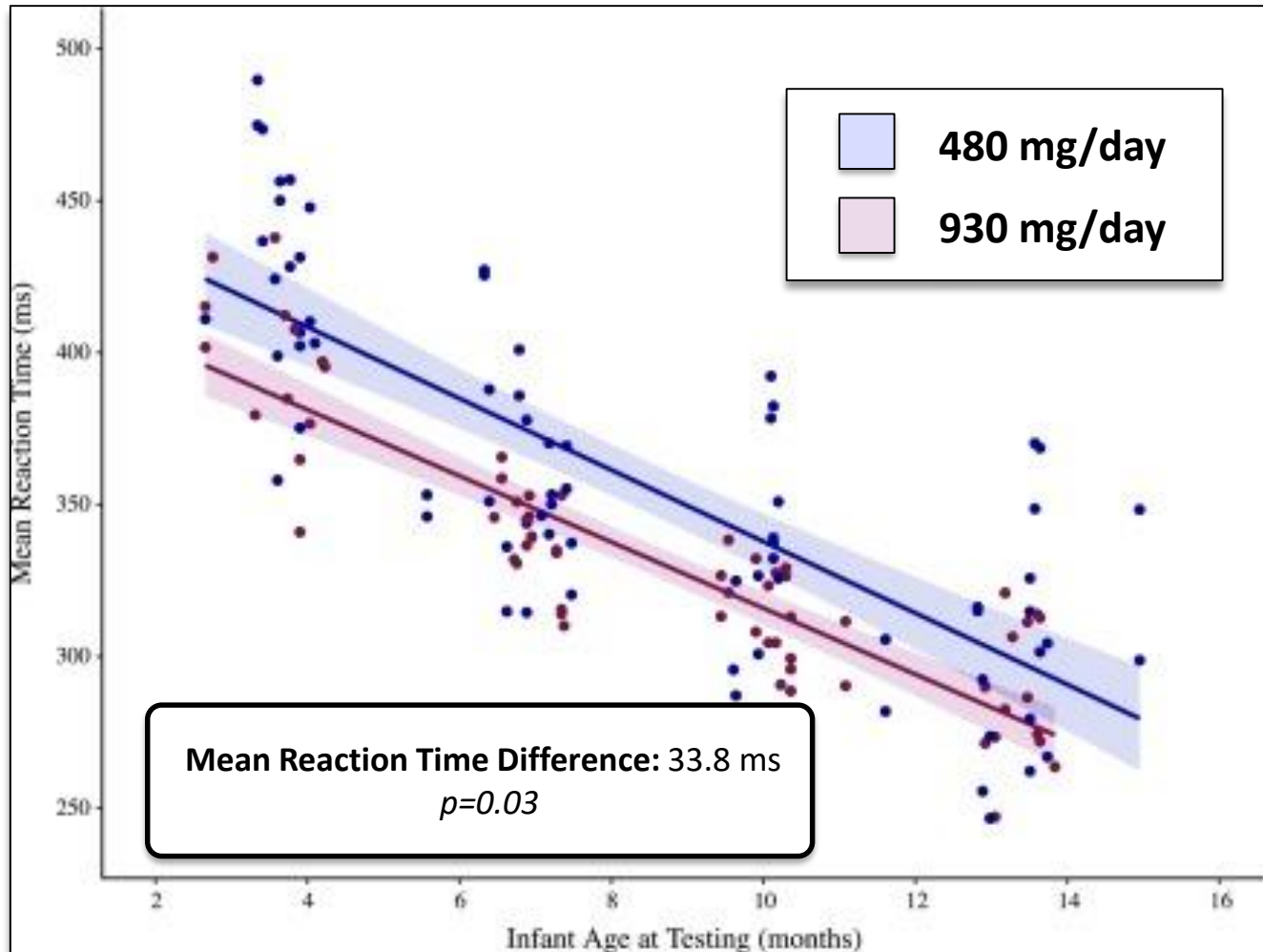
Predicts:

- Information processing speed later in childhood
- Childhood intelligent quotient (IQ)



Caudill MA, et al., *FASEB J* 2018; 32(4): 2172-2180

Maternal Choline Supplementation Improves Infant Reaction Time



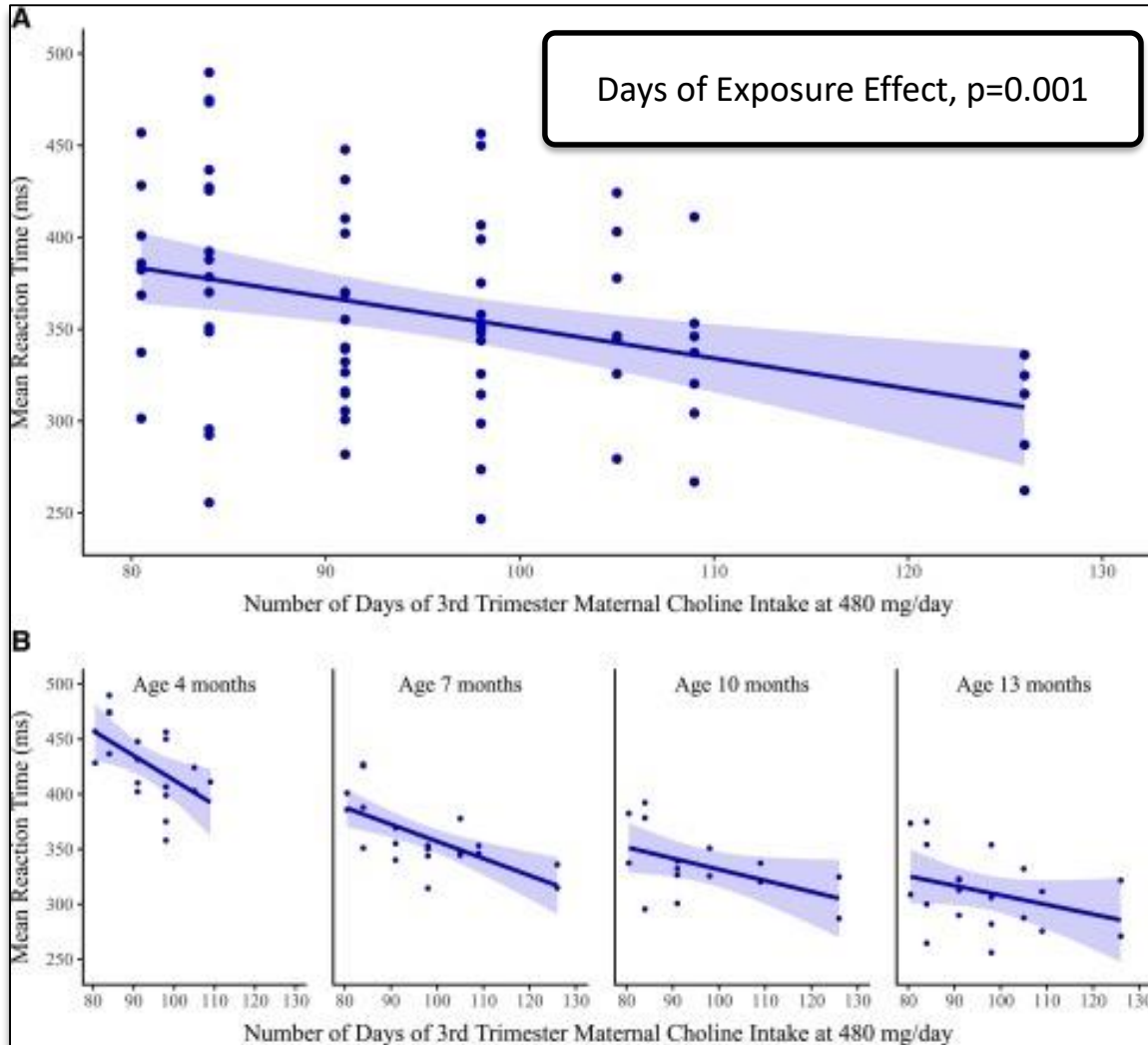
Results & Conclusions

- Infants born to high choline consuming mothers had significantly faster average reaction times compared to infants born to lower choline consuming mothers

Choline supplementation among women in their 3rd trimester of pregnancy improved information processing speed in their children

Caudill MA, et al., *FASEB J* 2018; 32(4): 2172-2180

Maternal Choline Supplementation Improves Infant Reaction Time

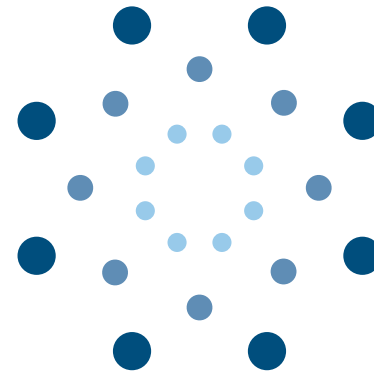


Results & Conclusions

- The number of days of prenatal exposure to choline was associated with a faster reaction time among infants born to low choline consuming mothers

“Even modest increases in typical maternal choline intake during pregnancy would be beneficial for infant information processing speed, with possible long-term benefits for offspring cognitive function throughout life”

Caudill MA, et al., *FASEB J* 2018; 32(4): 2172-2180



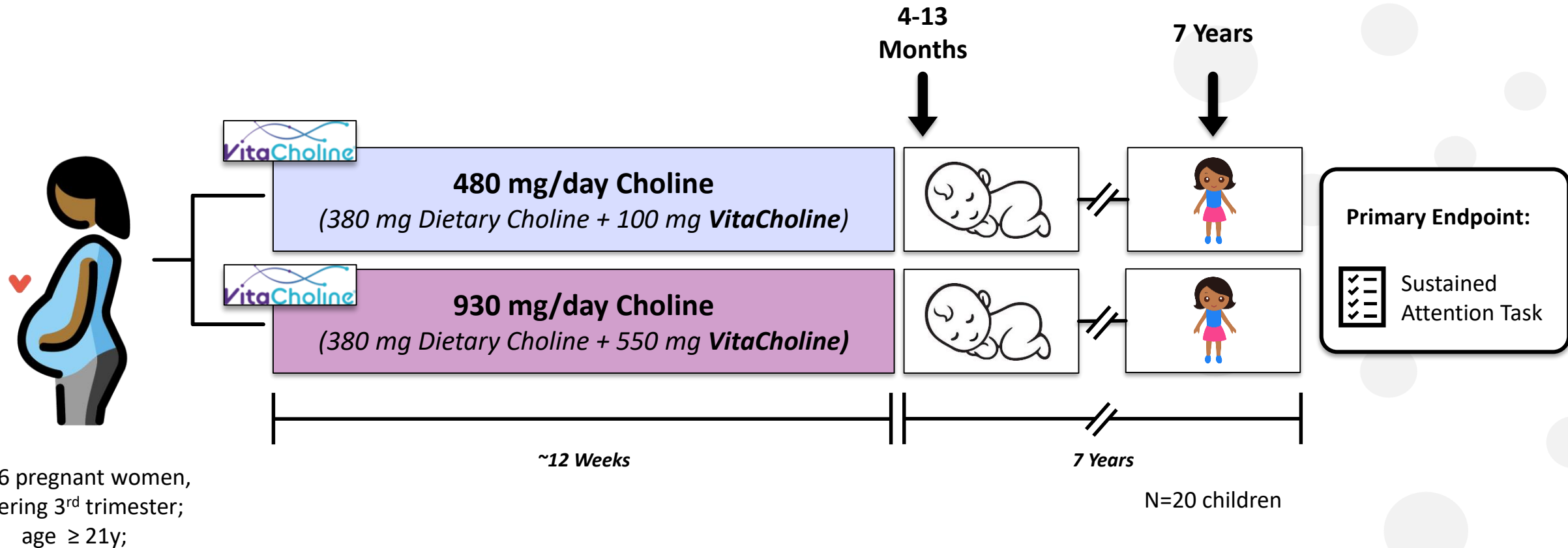
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Prenatal choline supplementation
improves child sustained attention:
A 7-year follow-up of a randomized
controlled feeding trial

Bahnfleth CL, et al. *FASEB J* 2022; 36(1): e22054

Does Maternal Choline Improve Baby's Brain Health Into Childhood?

Study Goal: To examine the long-term effects of maternal choline supplementation during pregnancy on offspring cognition

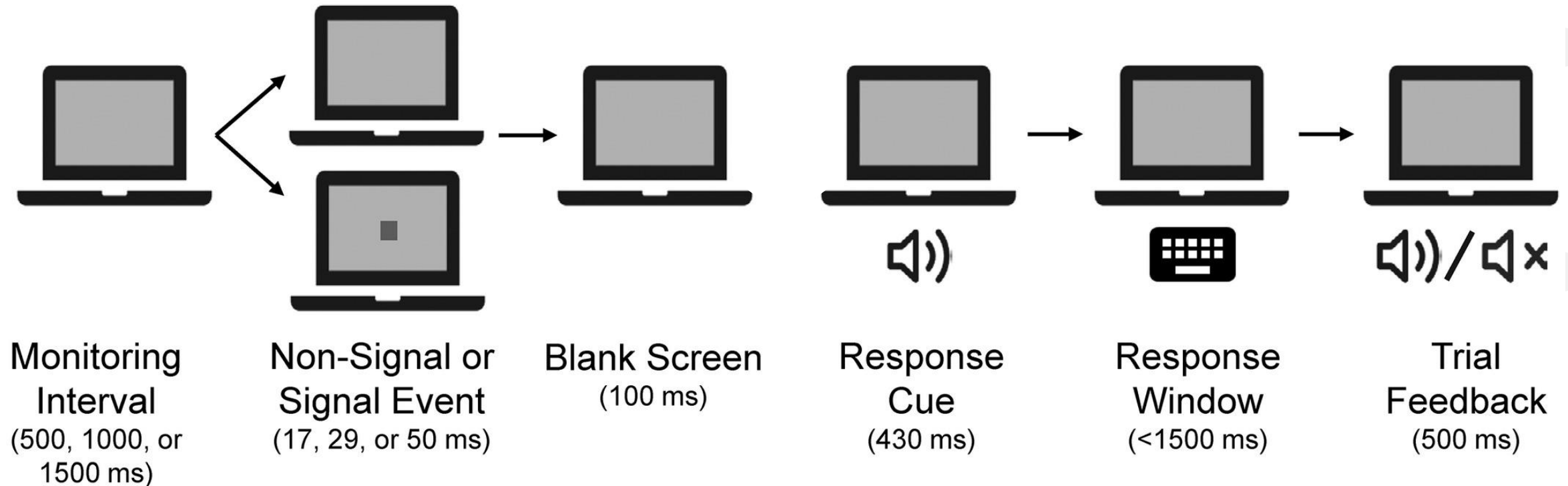


Bahnfleth CL, et al. *FASEB J* 2022; 36(1): e22054; All subjects also received OTC prenatal multivitamin, 200 mg DHA, supplemental K & Mg; Choline supplied as Choline Chloride

How Do You Measure Cognition in 7 Year Old Children?

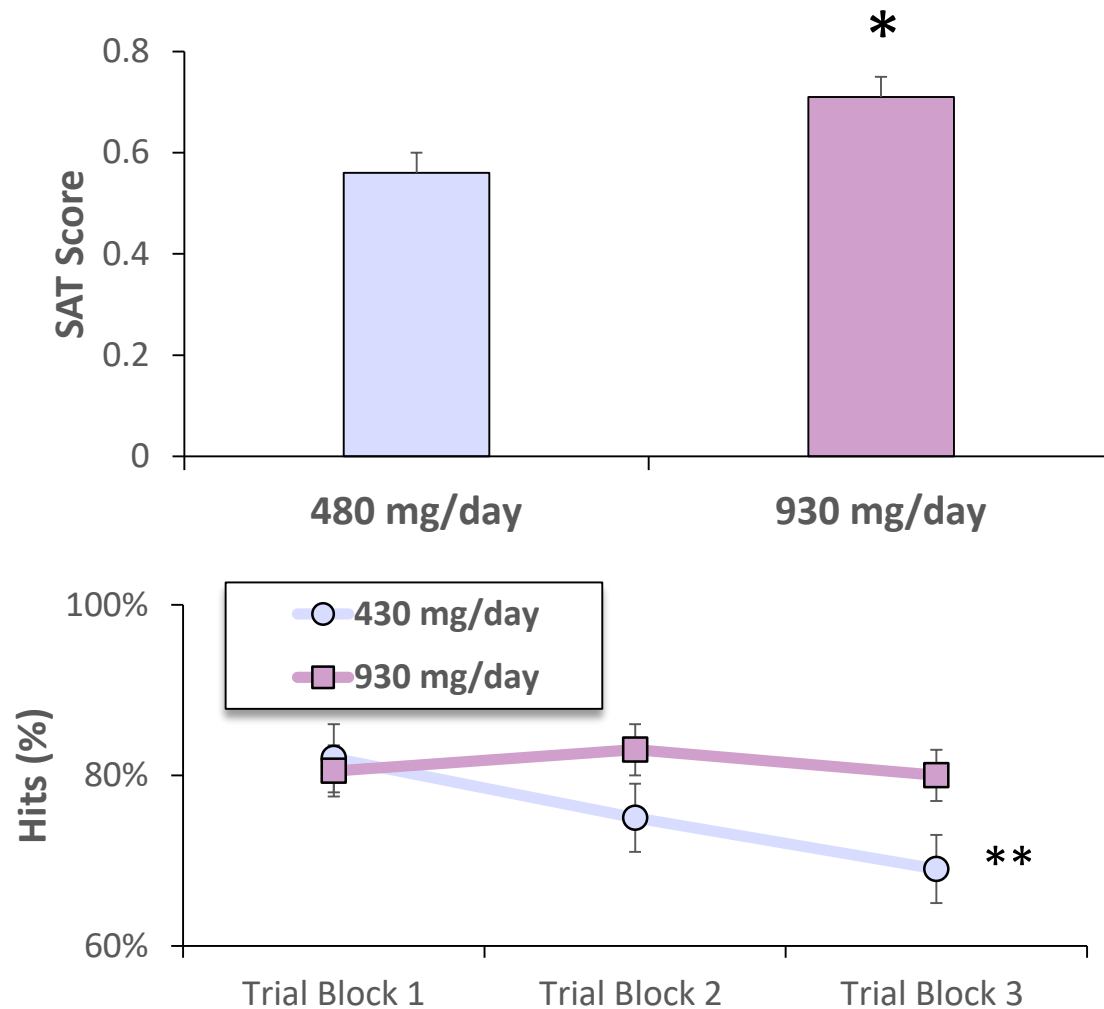
Primary Endpoint:
Sustained Attention Task

- **12 Minutes** (analyzed in 3 test blocks)
- **216 Tests x 3 Signal Durations** (17, 29, 50 ms)



Bahnfleth CL, et al. *FASEB J* 2022; 36(1): e22054.

Cognitive Benefits of Maternal Choline Supplementation Are Long-Term

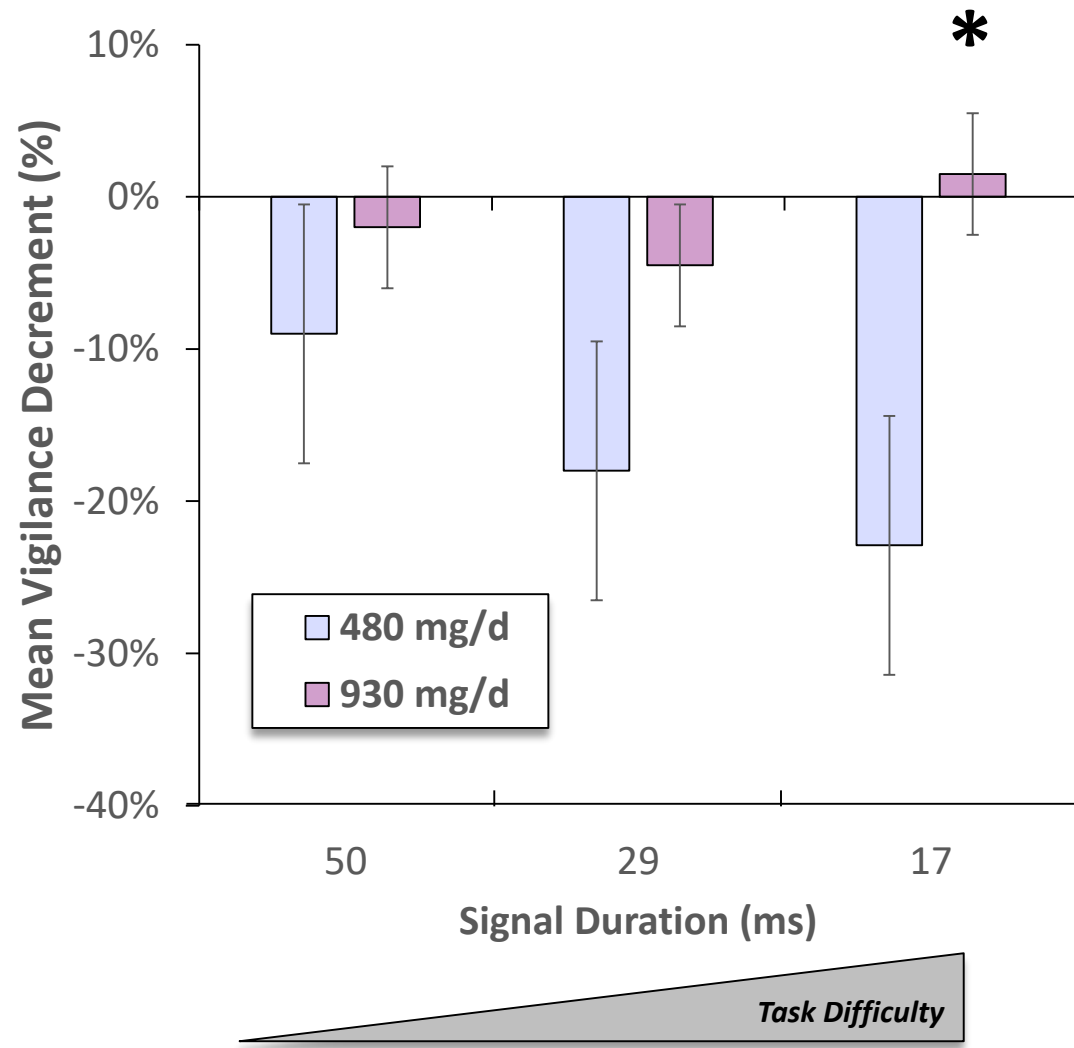


Results

- Children born to high choline consuming mothers **showed greater attention control and signal detection performance**
- Children born to high choline consuming mothers showed a **superior ability to maintain correct signal detections** across the 12-min session, indicating **improved sustained attention**

Adapted From: Bahnfleth CL, et al. *FASEB J* 2022; 36(1): e22054; *Significant difference between groups, $p < 0.05$; **Significant within-group trial time effect, $p < 0.05$

Maternal Choline Supplementation Prevented Attention Loss in Children



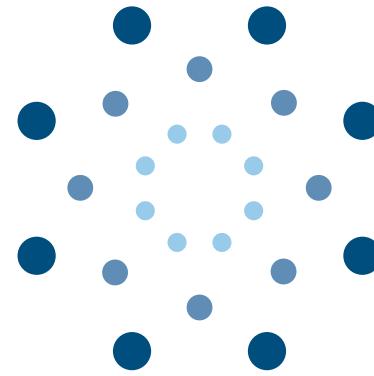
Results

- Children born to high choline consuming mothers **maintained their attention during the most difficult task**, while children born to lower-choline consuming moms showed **significant declines in attention**

Conclusions

- The **beneficial effects of maternal choline supplementation** during pregnancy for baby's attention **are present at age 7**
- Sustained attention (and attentional control more broadly) **contributes to a wide variety of higher cognitive functions such as problem-solving and working memory** and is **positively associated with school performance**

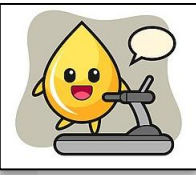
Adapted From: Bahnfleth CL, et al. *FASEB J* 2022; 36(1): e22054; *Significant difference between groups, $p < 0.05$ (Bonferroni corrected)



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How Else Can Choline Help Expecting Mothers?

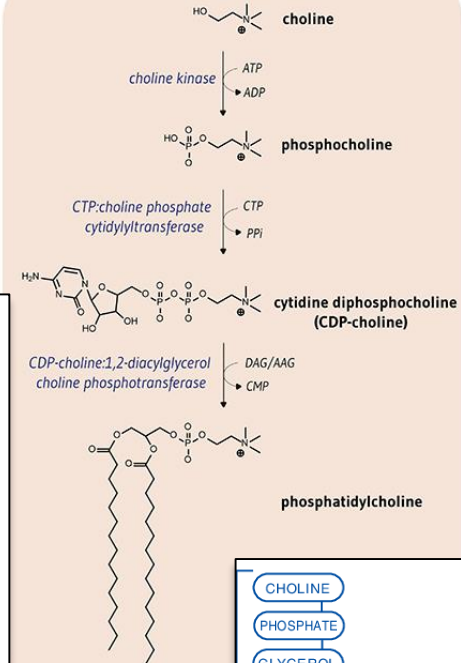
Choline Supports Lipid Metabolism



- Choline helps to generate **Phosphatidylcholine**, which has two key roles:
 - Acts as a structural component of **cell membranes**
 - Helps to **transport lipids** throughout the body to **support fat metabolism**
- Choline deficiency results in **organ damage** and **dysfunctional lipid metabolism**

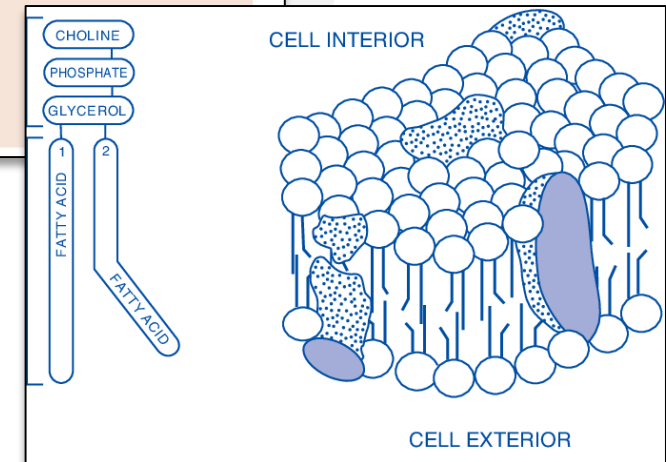
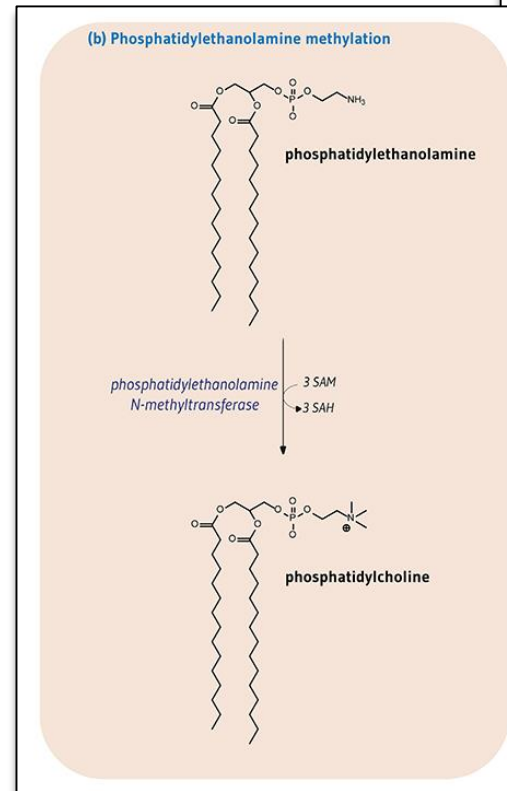
CDP Pathway

(a) The CDP-choline pathway



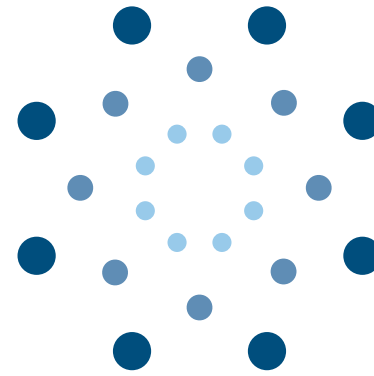
PEMT Pathway

(b) Phosphatidylethanolamine methylation



PC in cell membranes

Source: Linus Pauling Institute, Oregon State University; <https://lpi.oregonstate.edu/mic/other-nutrients/choline>; Alberts et al. (eds), Molecular Biology of the Cell, 1996.



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Choline metabolome response to prenatal choline
supplementation across pregnancy:
A randomized controlled trial

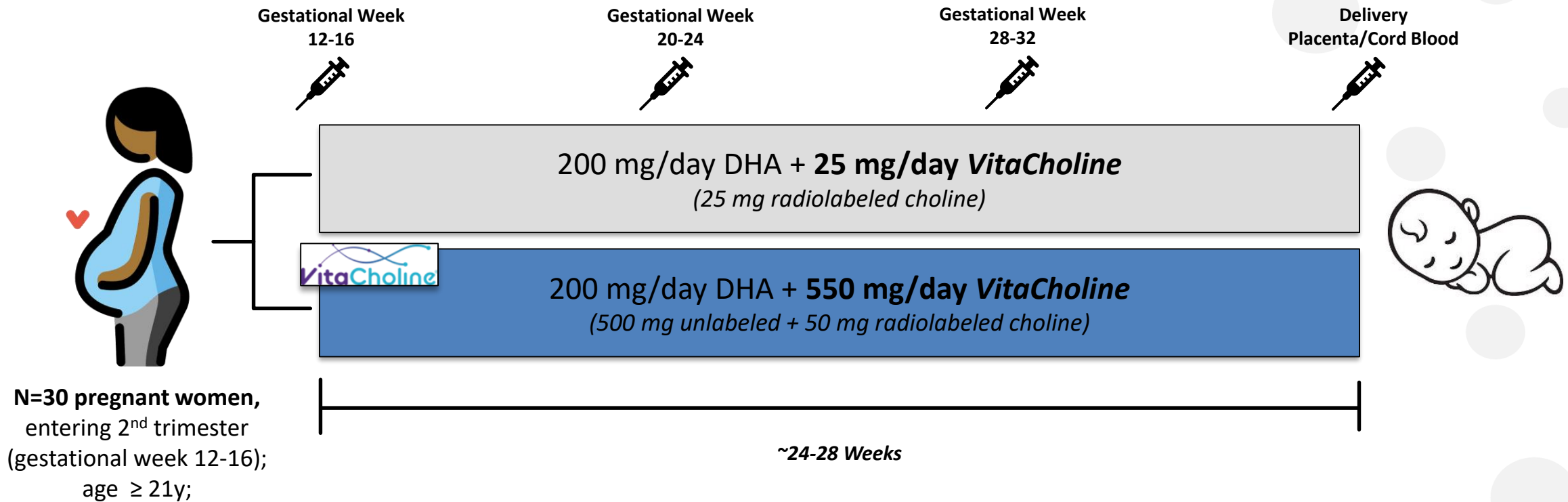
Taesuwan S, et al., *FASEB J* 2021; 35(12): e22063.

Does Maternal Choline Supplementation Improve Choline Status?

Study Goal: To investigate the effect of prenatal choline supplementation on maternal and fetal biomarkers of choline metabolism among free-living pregnant persons

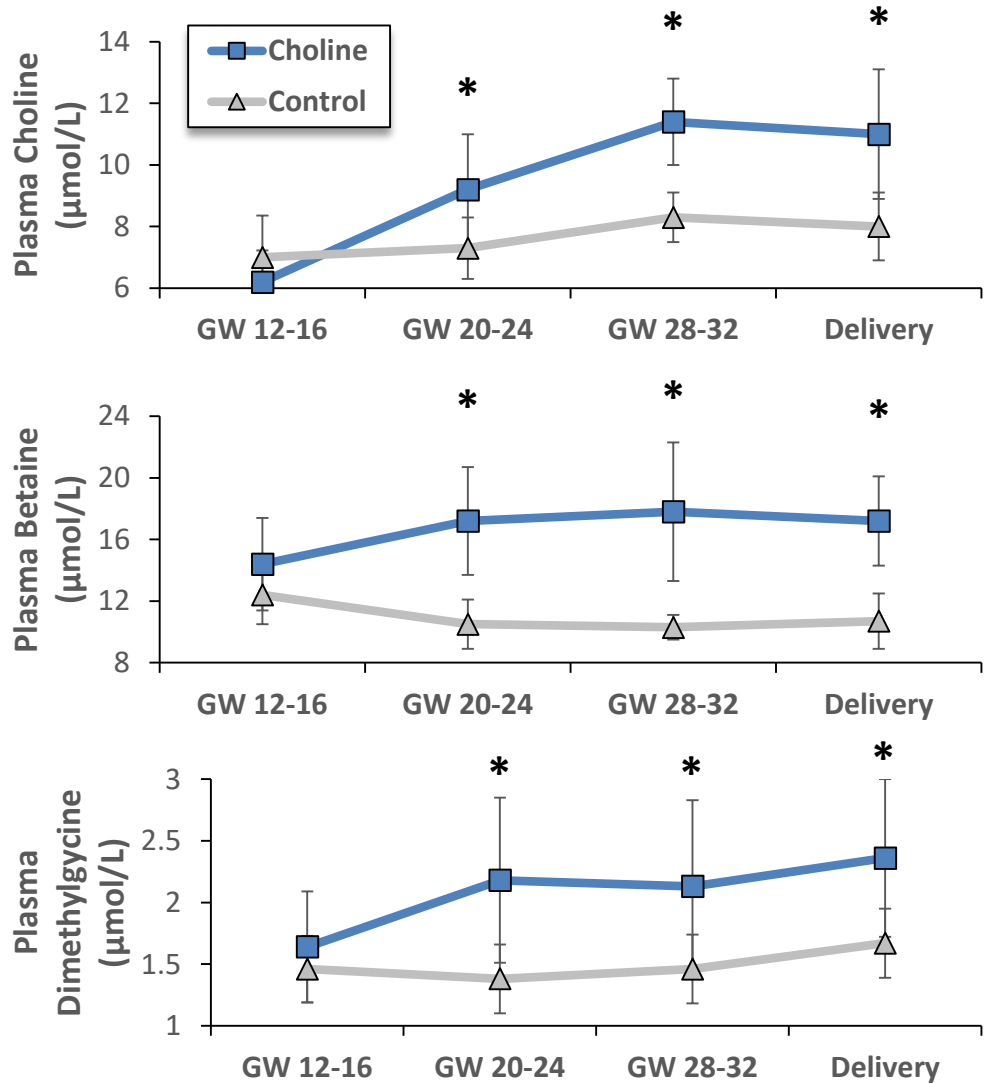
Primary Endpoints:

 Markers of Choline Status



Taesuan S, et al., *FASEB J* 2021; 35: e22063. All subjects also received OTC prenatal multivitamin, Choline supplied as Choline Chloride in a grape juice cocktail

Maternal Choline Metabolome Is Responsive to Choline Supplementation



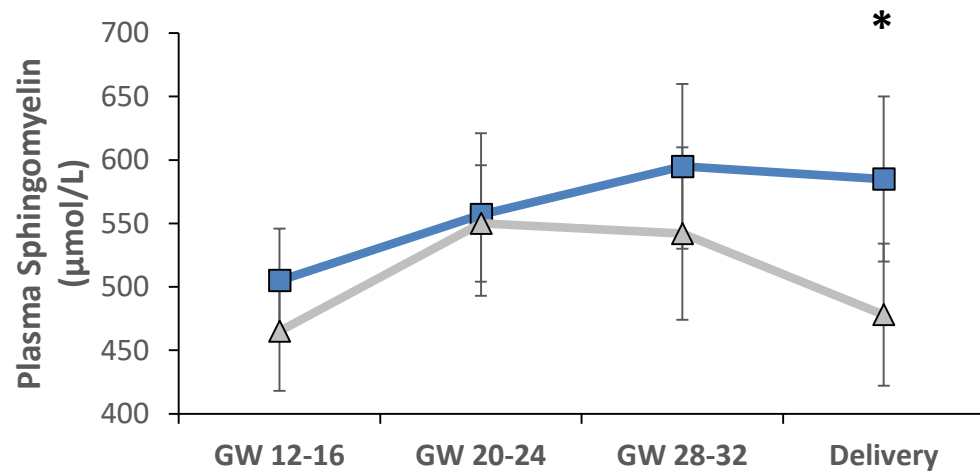
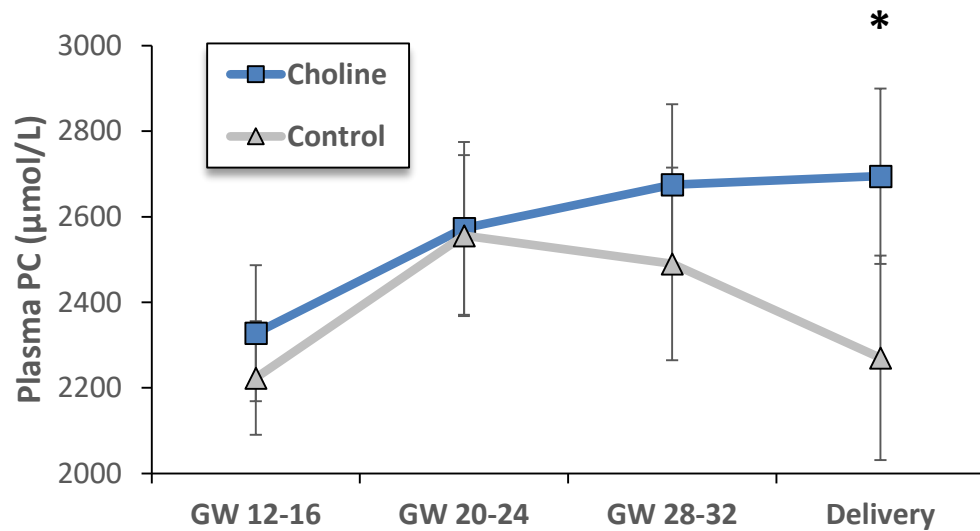
Results & Conclusions

- **VitaCholine** supplementation during pregnancy significantly **increased maternal choline concentrations** in plasma
- **VitaCholine** supplementation during pregnancy significantly **increased the concentration of key one-carbon nutrients** in plasma

VitaCholine is bioavailable in pregnant women

Taesuwan S, et al., *FASEB J* 2021; 35: e22063. Mean ± 95% CI; *Significant difference between groups, p<0.05

Maternal Choline Metabolome Is Responsive to Choline Supplementation



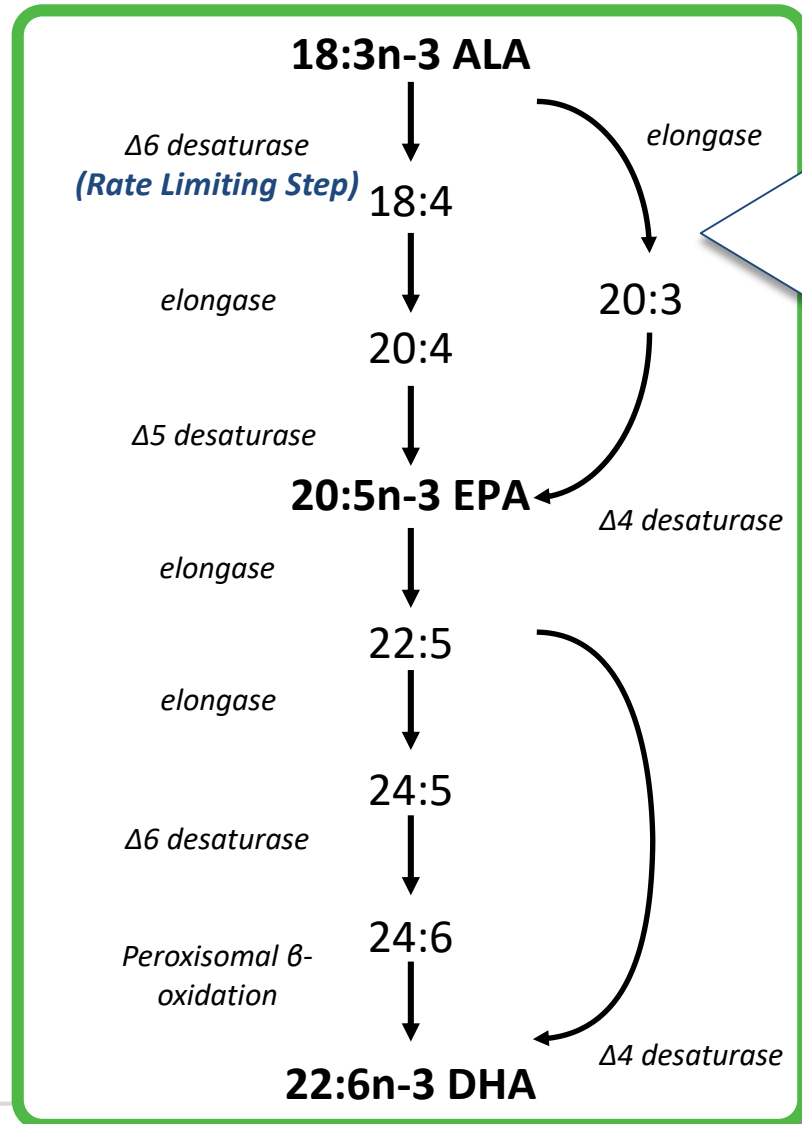
Results & Conclusions

- **VitaCholine** supplementation increased markers of the lipid soluble metabolome

VitaCholine helps maintain normal lipid metabolism during pregnancy

Taesuwan S, et al., *FASEB J* 2021; 35: e22063. Mean ± 95% CI; *Significant difference between groups, p<0.05

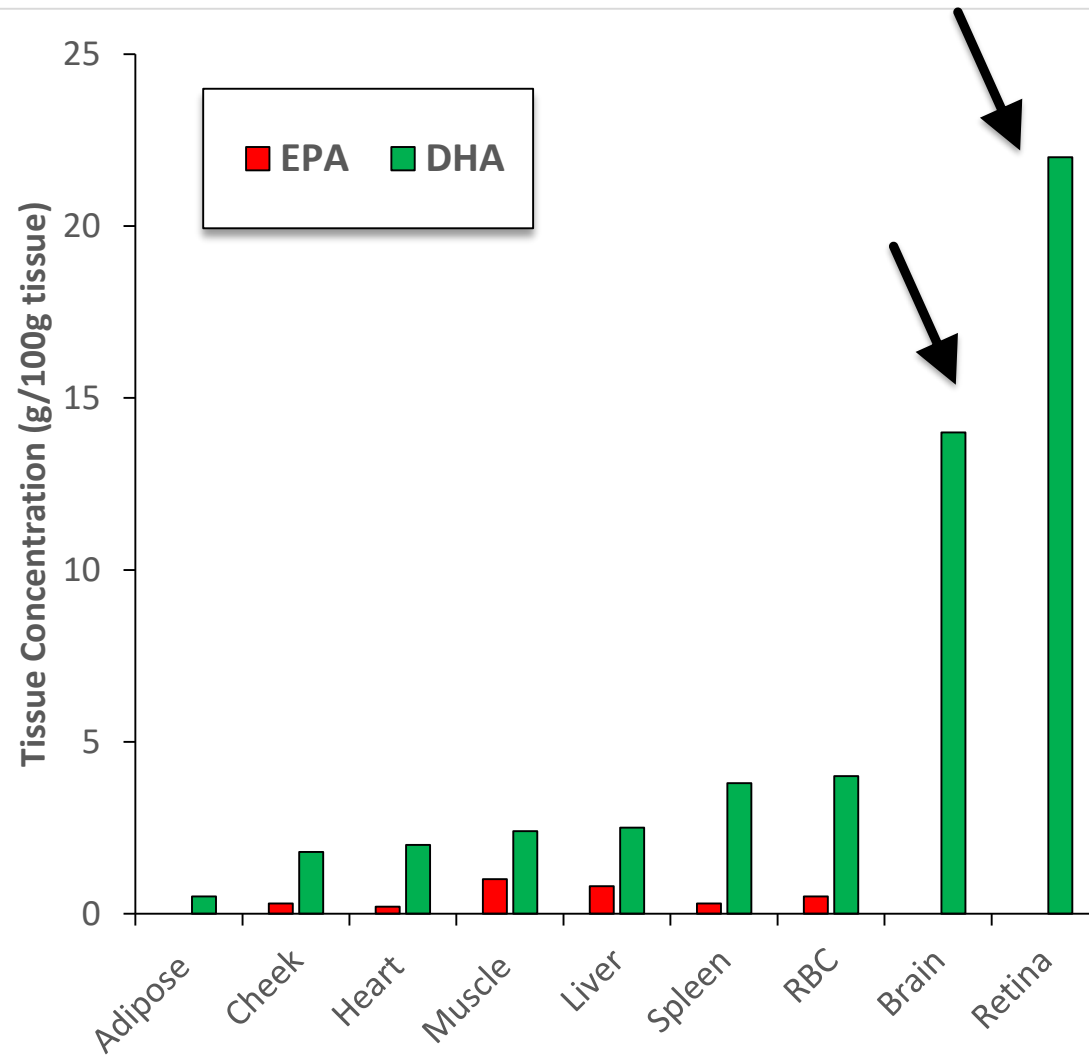
What Are Omega-3 Fatty Acids?



- Alpha-Linolenic Acid (ALA) is the omega-3 fatty acid found in flaxseed oil
- According to the Institute of Medicine, ALA's primary role is to serve as a **precursor for EPA and DHA**
- The conversion of ALA to EPA is **0.01-8.0%**, and may be **even less** for DHA

Adapted from: Flock et al., *Nutr Rev* 2013; 71(10): 692-707.

DHA is the Primary Omega-3 Found in the Brain and Eye



- **DHA accumulates primarily in the brain and retina** where it plays important structural and functional roles
- **DHA is critical for brain and eye health** throughout the lifespan

Arterburn et al., *Am J Clin Nutr* 2006; 83(6 suppl): 1476S-1476S; RBC = Red Blood Cell

Experts Recommend DHA During Pregnancy



Expert Group	DHA Intake Recommendation	Source
American Academy of Pediatrics (AAP)	200-300 mg/day DHA	American Academy of Pediatrics. Pediatrics 2012; 129(3): e827-e841.
Australian National Health and Medical Research Council (NHMRC)	800 mg/day DHA + 100 mg/day EPA	https://www.health.gov.au/resources/pregnancy-care-guidelines/part-c-lifestyle-considerations/nutrition-and-physical-activity#112-nutritional-supplements
European Food Safety Authority (EFSA)	100-200 mg/day DHA	EFSA Journal 2010; 8(3): 1461
Food & Agriculture Organization of the United Nations (FAO) World Health Organization (WHO) Joint Expert Consultation	200 mg/day DHA	https://www.fao.org/3/i1953e/i1953e00.pdf
Global Organization for EPA and DHA Omega-3 (GOED)	300 mg/day DHA	https://goedomega3.com/intake-recommendations

Expert groups generally recommend **200-300 mg/day of DHA for pregnant women**

Additional recommendations outlined in: Zhang Z, et al., *Nutrients* 2018; 10(4): 416. doi: 10.3390/nu10040416.

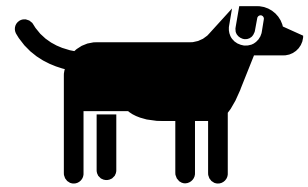
Does Maternal DHA Intake Impact Puppy Trainability?

Study Goal: To investigate the effect of prenatal choline supplementation on maternal and fetal biomarkers of choline metabolism among free-living pregnant persons

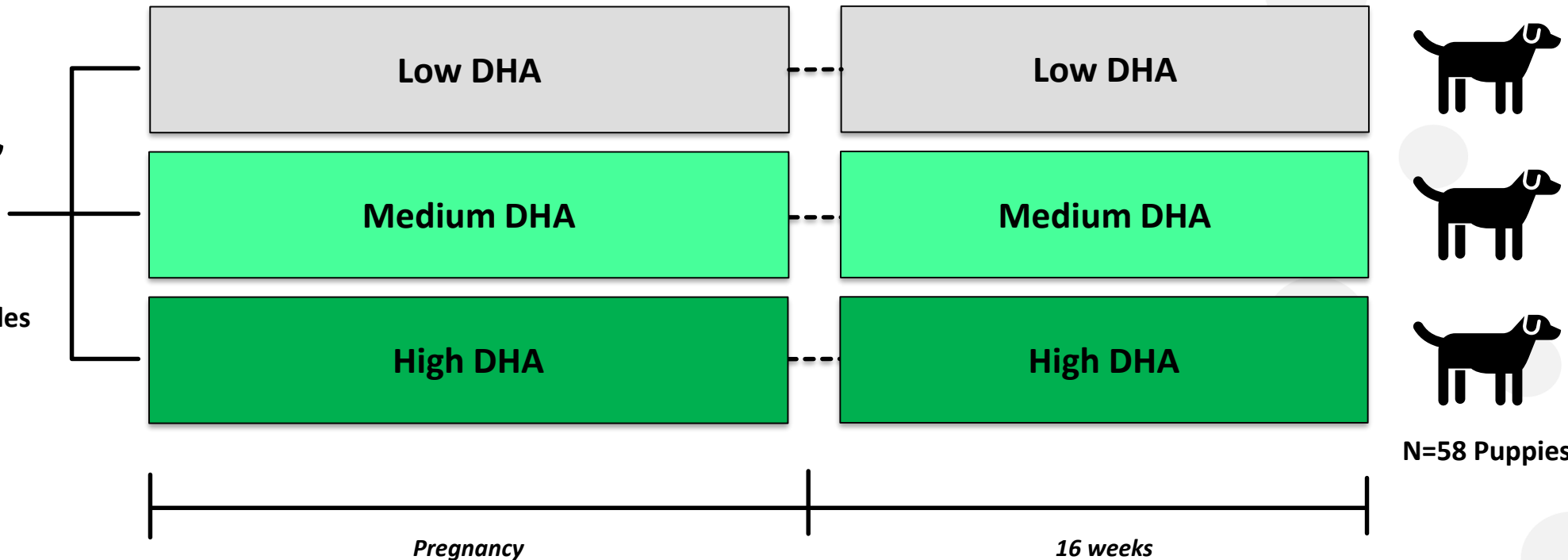
Primary Endpoints:



Puppy Trainability
(Standard T-Maze)

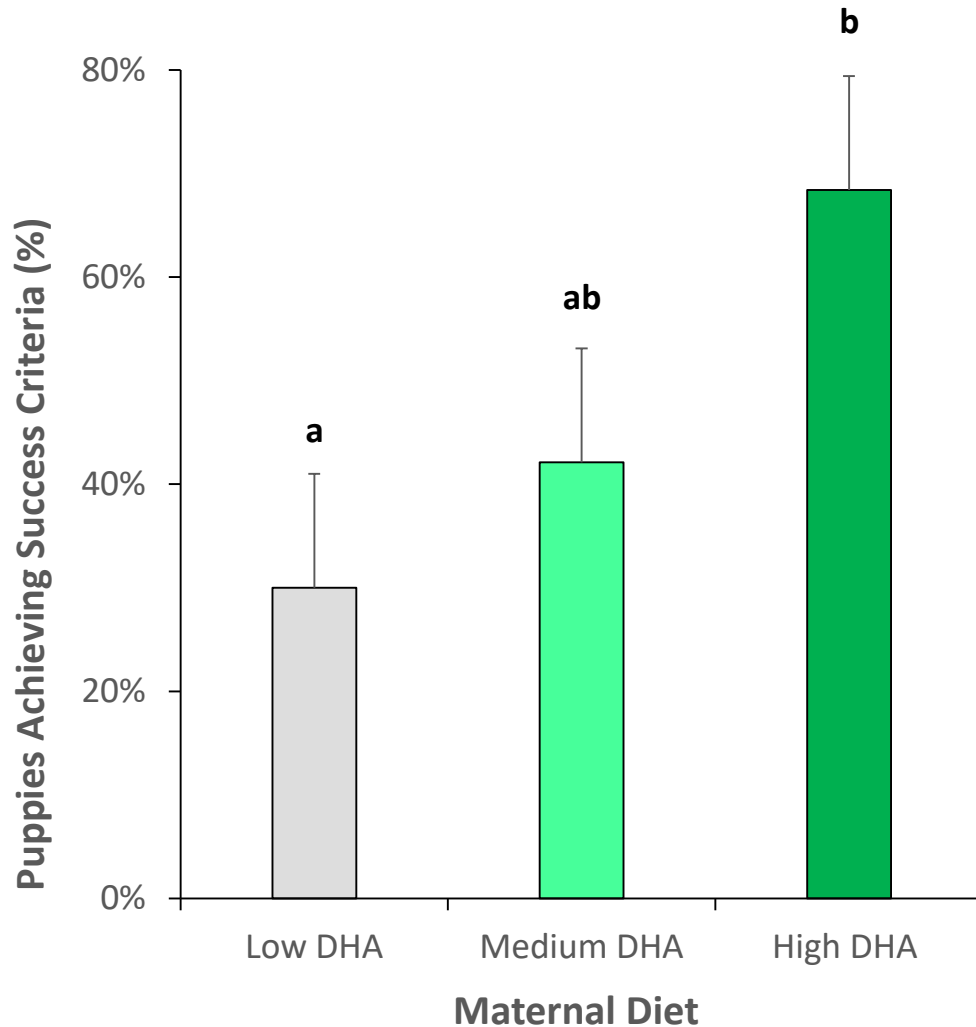


N=28 Female Beagles



Kelley RL, et al; <https://breedingbetterdogs.com/article/nutrition-and-dha>

Maternal DHA Intake Improves Puppy Trainability



Results & Conclusions

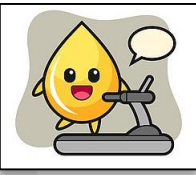
- Significantly more puppies born to high DHA consuming mothers achieved training success compared to those consuming lower DHA diets
 - 68.4% vs 30%

Maternal DHA intake improves neurological function and trainability in dogs

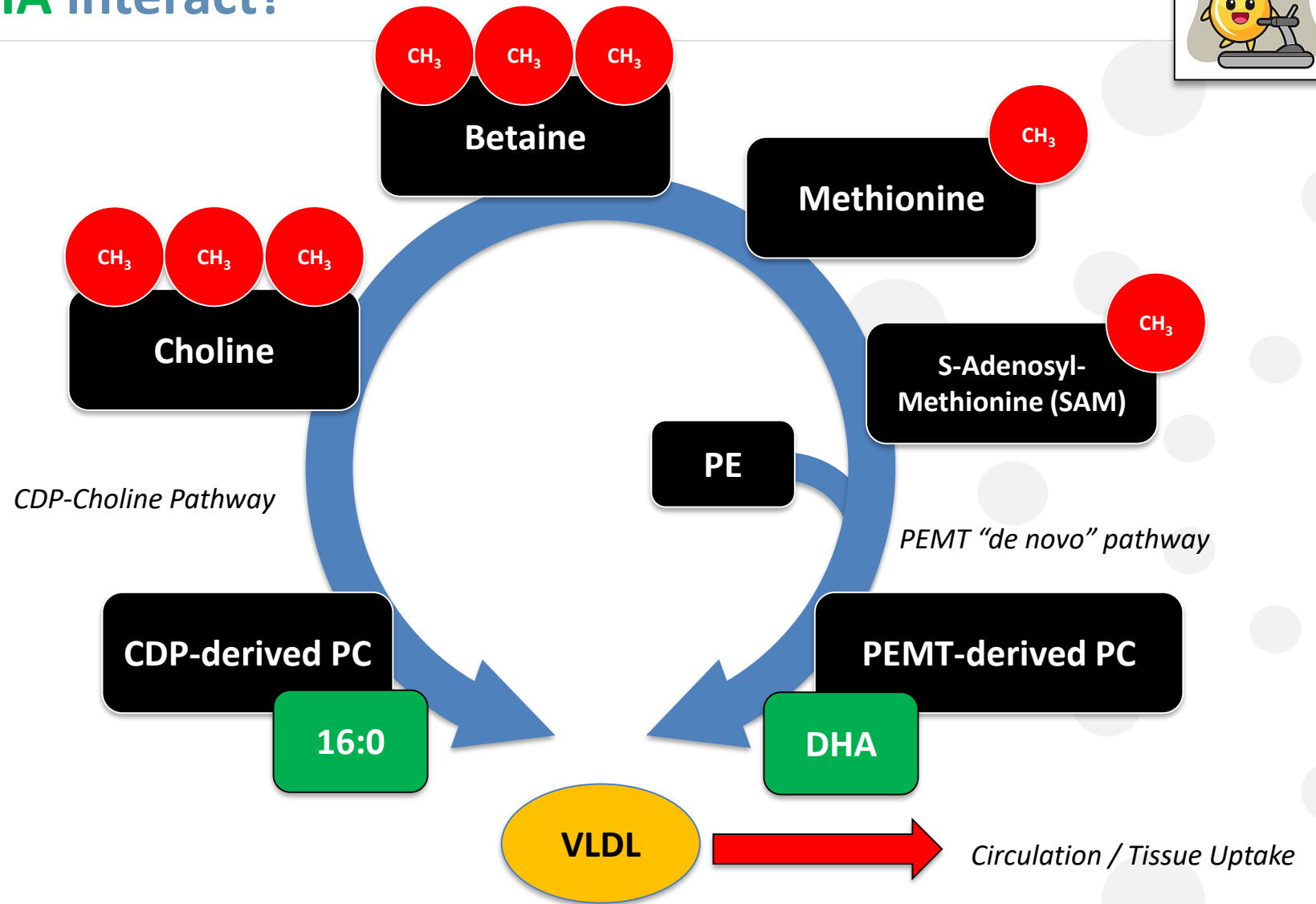
Kelley RL, et al; <https://breedingbetterdogs.com/article/nutrition-and-dha>; Success Criteria = 8/10 correct trials for 2 consecutive sessions;

Groups with different subscripts are significantly different, $p < 0.05$

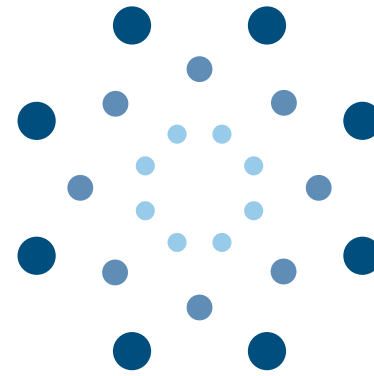
How Do Choline and DHA interact?



- The PEMT pathway uses methyl groups from choline to generate PC-DHA
- If we added more choline (and more methyl groups) through the diet, would we increase DHA status?



Adapted From: Yan J, et al. *Am J Clin Nutr* 2013; 98: 1459-1467; PC = Phosphatidylcholine; PE = Phosphatidylethanolamine; PEMT = Phosphatidylethanolamine-N-methyltransferase



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Prenatal choline supplementation improves biomarkers of maternal docosahexaenoic acid status among pregnant participants consuming supplemental DHA: A randomized controlled trial

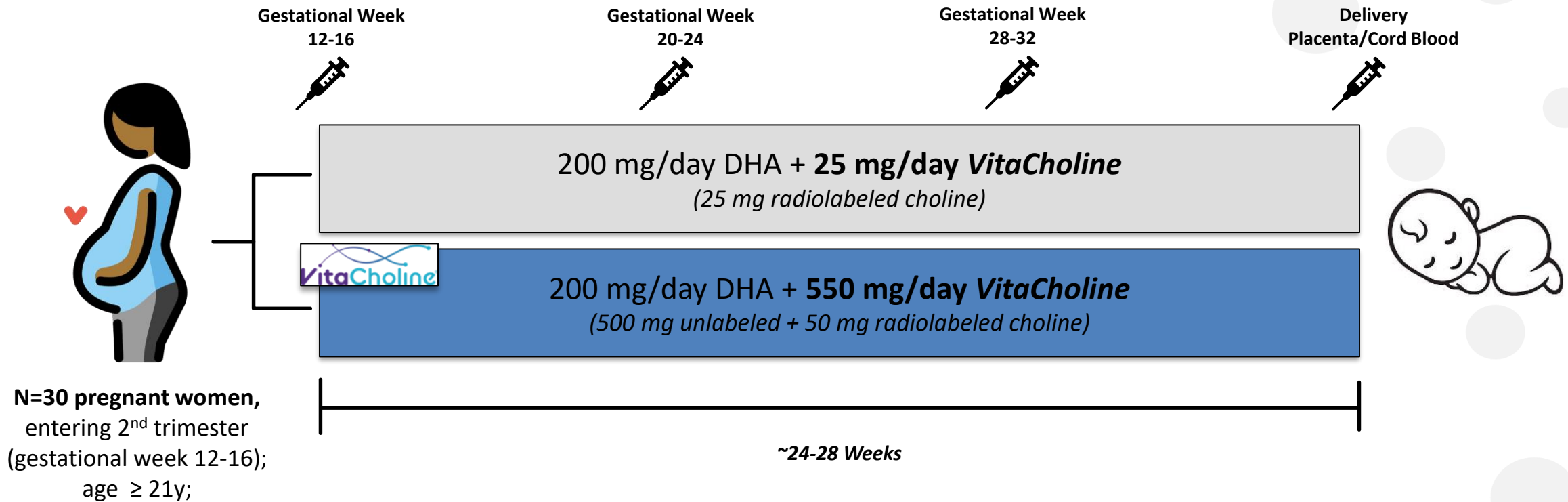
Klatt KC, et al. (*submitted*)

Does Maternal Choline Supplementation Improve DHA Status?

Study Goal: To determine the effect of prenatal choline supplementation on biomarkers of DHA status among pregnant participants consuming supplemental DHA

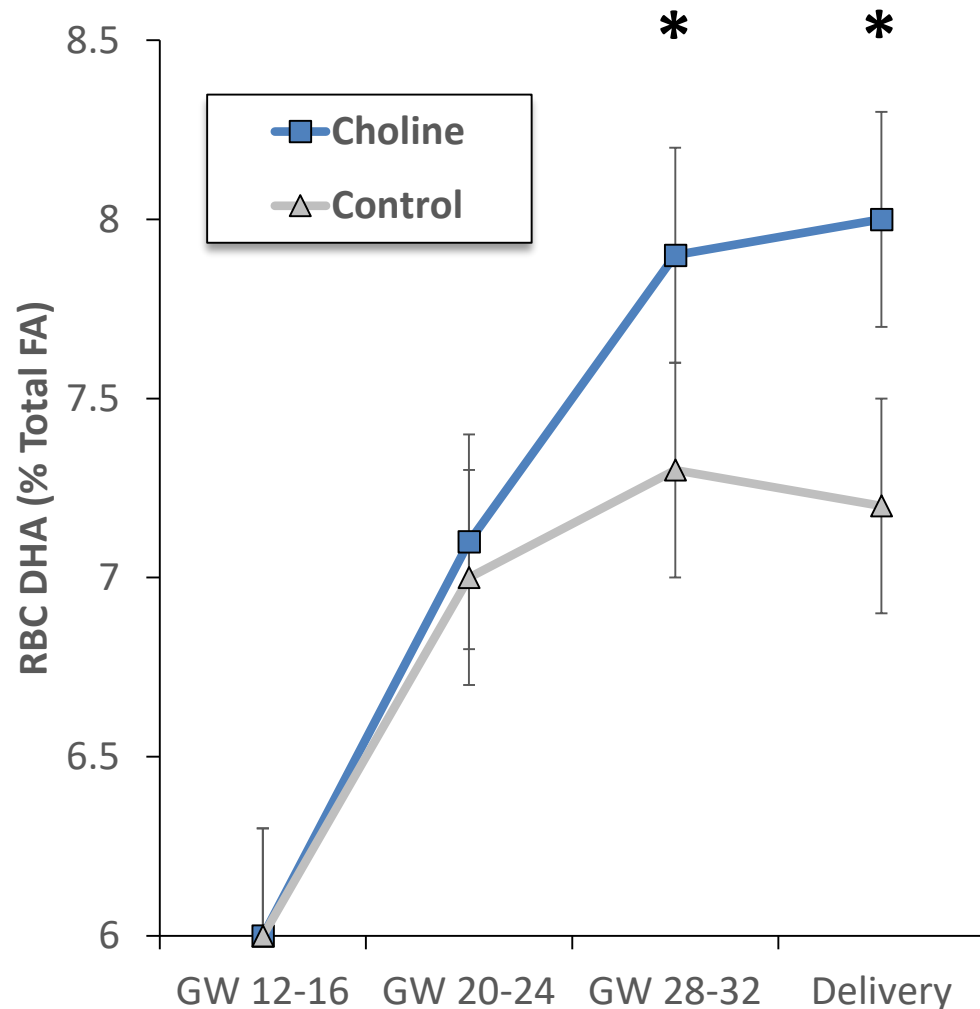
Primary Endpoints:

 Markers of DHA Status



Klatt KC, et al., (submitted); All subjects also received OTC prenatal multivitamin, Choline supplied as Choline Chloride in a grape juice cocktail

Choline Supplementation During Pregnancy Improves DHA Status



Results & Conclusions

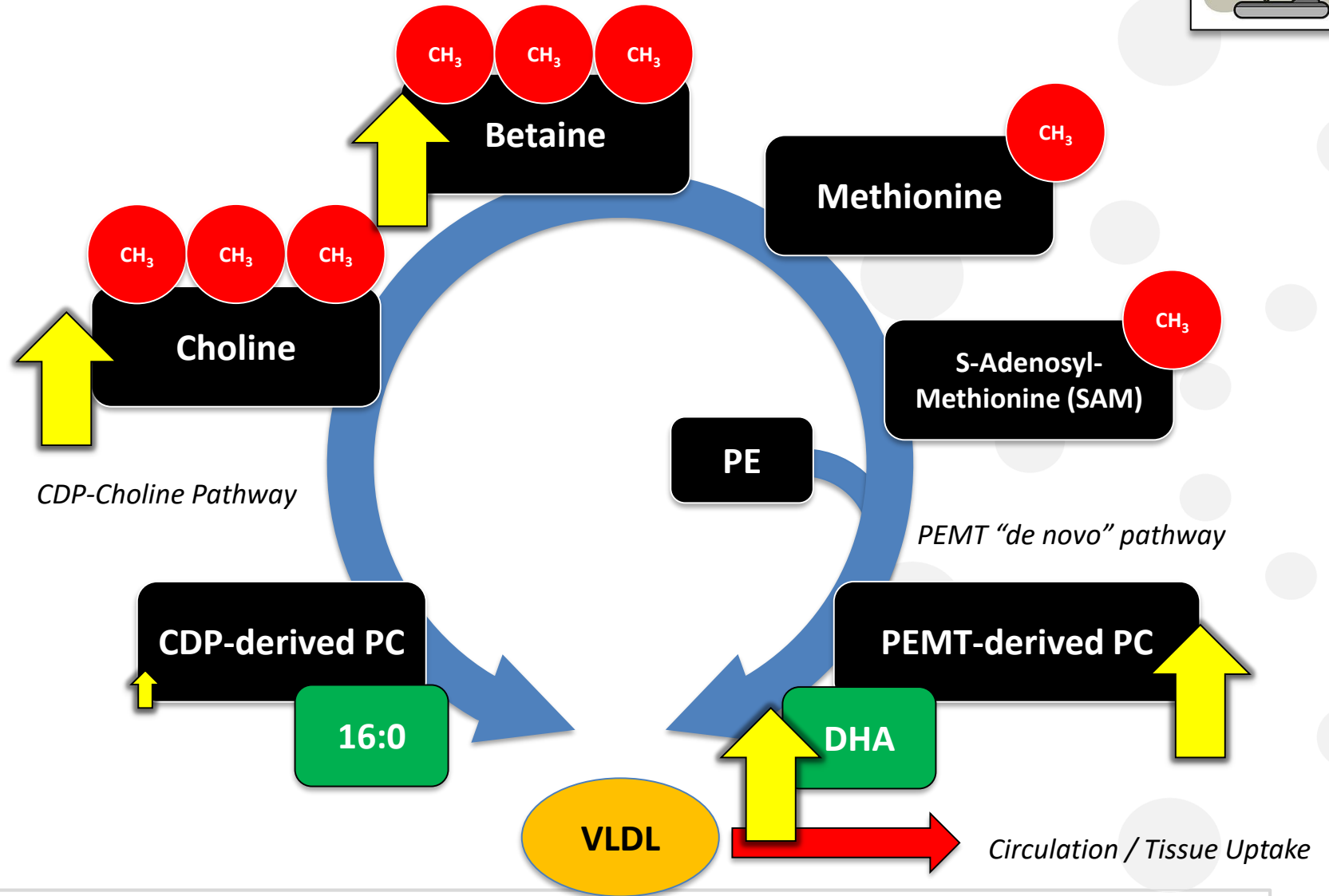
- Maternal supplementation of **VitaCholine** and DHA increases mother's DHA status **better than DHA alone**
- Adequate maternal DHA status during pregnancy is **critical to ensure proper supply of nutrients to the developing baby**

Klatt KC, et al., (submitted); Mean ± 95% CI; *Significant difference between groups, p<0.05

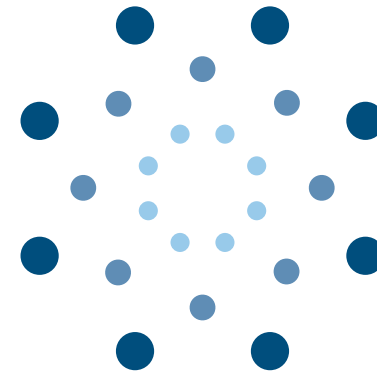
Choline Supplementation During Pregnancy Improves DHA Status



- Supplementation with **VitaCholine** during pregnancy with increases markers of **Choline status**
- Supplementation with **VitaCholine + DHA** during pregnancy increases DHA status better than DHA alone



Taesuwan S, et al., *FASEB J* 2021; 35: e22063; Klatt KC, et al., (submitted); PC = Phosphatidylcholine; PE = Phosphatidylethanolamine; PEMT = Phosphatidylethanolamine-N-methyltransferase



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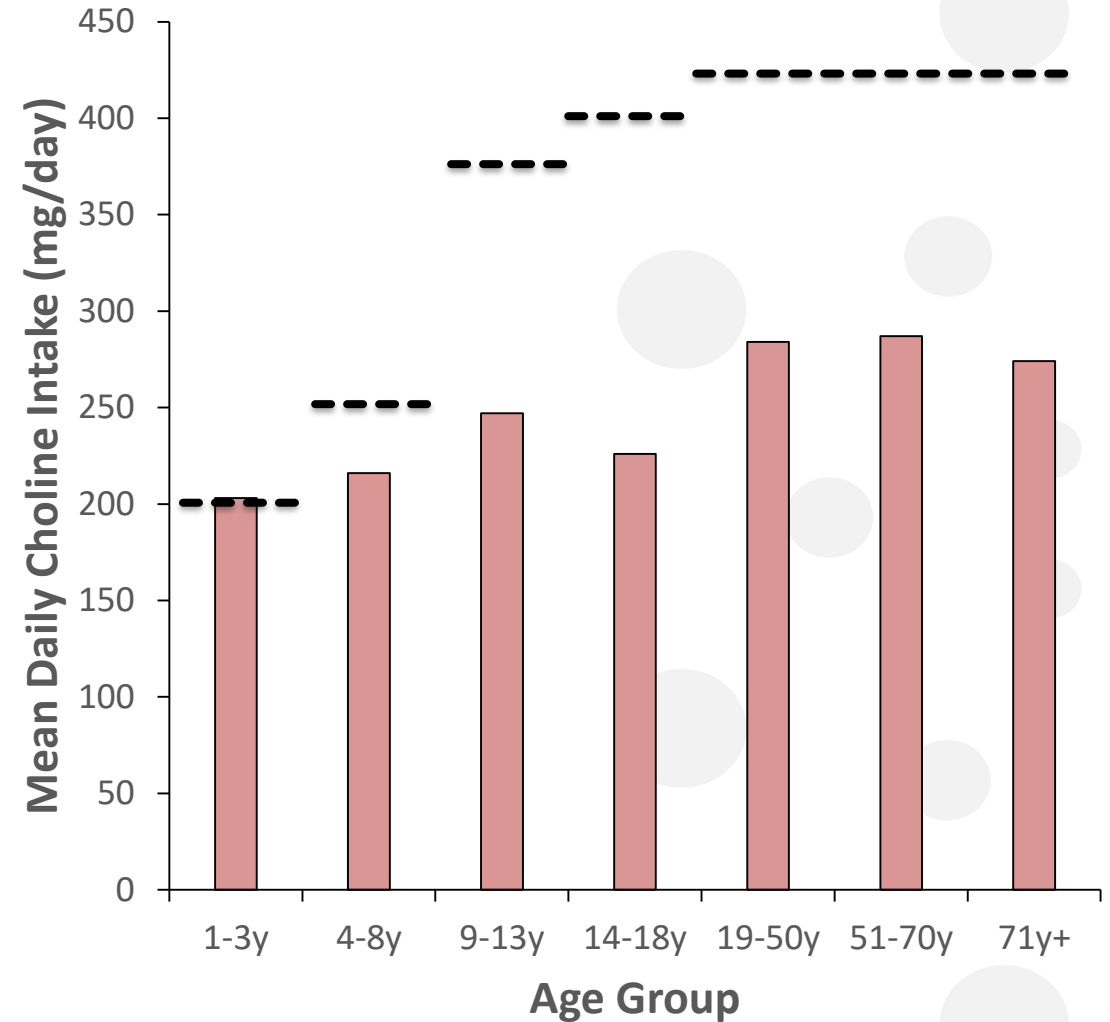
Are We Getting Enough Choline?

Are We Getting Enough Choline?



Women at nearly every age group **do not get enough choline** in their diet

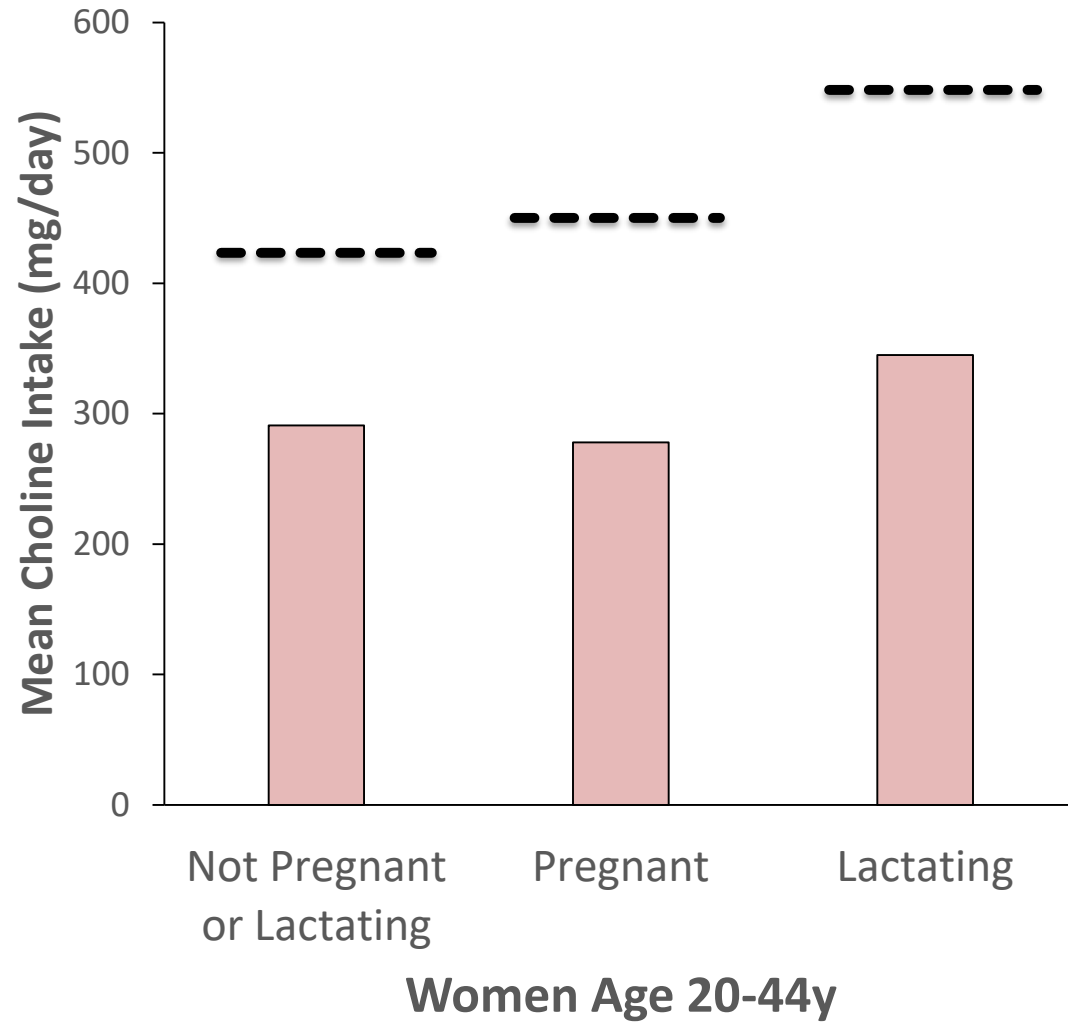
Just **6% of Adult Women** in the United States get enough choline in their diet



Source: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/usual/Usual_Intake_gender_WWEIA_2015_2018.pdf; Usual nutrient intake from food & beverages; n=15,210

----- = Adequate Intake (AI) for group

Choline Inadequacy is Common Among **Pregnant & Lactating Women**



Less than 1 in 20 pregnant (4%) or lactating (3%) women age 20-44y consume adequate amounts of choline

Source: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/usual/Usual_Intake_Pregnancy_Lactation_Status_WWEIA_2015_2018.pdf; Usual nutrient intake from food & beverages;

----- = Adequate Intake (AI) for group; n=2,063

Choline Inadequacy is Recognized by the Dietary Guidelines



*“Adequate intake of choline during [pregnancy & lactation] helps to both **replenish maternal stores** and **support the growth and development** of the child’s brain and spinal cord.”*

“Many prenatal supplements do not contain choline or only contain small amounts inadequate to meet recommendations.”



Source: 2020-2025 Dietary Guidelines for Americans; <https://www.dietaryguidelines.gov/>

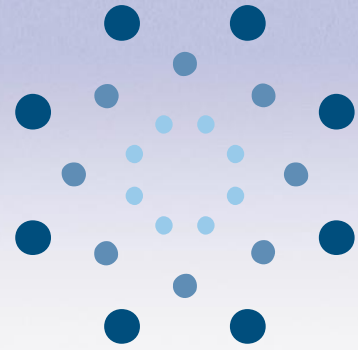
Summary: Maternal Choline Supplementation Benefits Baby



- **Choline** is an essential nutrient that **helps support the growth and development of the brain and eyes**
- Maternal **VitaCholine** supplementation provides significant brain health benefits for baby:
 - **VitaCholine** supplementation **significantly improves cognitive processing speeds in infants**
 - **VitaCholine** supplementation **improved sustained attention – at age 7!**
 - **VitaCholine** supplementation **improved attention control – at age 7!**
- Supplemental **VitaCholine** is **bioavailable** in expecting mothers
- Maternal **VitaCholine + DHA** supplementation **improves DHA status** better than supplementing DHA alone
- Improving DHA intake has been shown to improve trainability in canine models
- Expert groups are calling on manufacturers to **increase the amount of choline during pregnancy**

VitaCholine provides innovative benefits for both mom and baby!





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Solve Today. Shape Tomorrow.



Experts Are Calling to Increase Choline Content in Maternal Diets



“Prenatal vitamins only contain 0–55 mg of choline, leaving the majority of pregnant and lactating women without enough dietary choline to protect the health and development of their babies.”

The AMA supports *“evidence-based amounts of choline in all prenatal vitamins”*

Choline is a *“key nutrient that supports neurodevelopment”*

Pediatricians should prioritize *“public policies that ensure the provision of adequate nutrients and healthy eating”* to help *“ensure that all children have an early foundation for optimal neurodevelopment, a key factor in long-term health.”*



American Academy
of Pediatrics

Source: <https://www.ama-assn.org/sites/ama-assn.org/files/corp/media-browser/public/hod/a17-reference-committee-reports.pdf>

<https://www.ama-assn.org/delivering-care/public-health/ama-backs-global-health-experts-calling-infertility-disease>; Schwartzberg SJ, et al; *Pediatrics* 2018; 141(2): e20173716.