Making Animals Smarter: Nutrition & Cognition

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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.

Animal Studies Connect Maternal Choline Intake and Offspring Cognition

<table>
<thead>
<tr>
<th>Citation</th>
<th>Animal</th>
<th>Choline Intervention</th>
<th>Cognition Assessment</th>
<th>Offspring Age at Assessment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meck WH et al., <em>Dev Psychobiol</em> 1988; 21: 339-353.</td>
<td>Pregnant Rats</td>
<td>2d prior to conception through birth</td>
<td>• 12 &amp; 18 arm radial arm maze</td>
<td>60 days</td>
<td>• ↑ Visuospatial Memory</td>
</tr>
<tr>
<td>Glenn MJ et al., <em>Brain Res</em> 2008; 1237: 110-123.</td>
<td>Pregnant Rats</td>
<td>E12-E17</td>
<td>• Open field exploration • Novel object exploration • Hippocampal Plasticity (IHC, Stereology)</td>
<td>1 month 24 months</td>
<td>• Associated w/exploratory behavior • ↑ Hippocampal plasticity</td>
</tr>
<tr>
<td>Meck WH, Williams CL; <em>Brain Res</em> 1999; 118: 51-59.</td>
<td>Pregnant Rats</td>
<td>E11-E18</td>
<td>• 12 arm radial arm maze</td>
<td>120 days</td>
<td>• ↑ Accuracy</td>
</tr>
<tr>
<td>Meck WH, Williams CL; <em>Neuroreport</em> 1997; 8: 3053-3059.</td>
<td>Pregnant Rats</td>
<td>E12-E17</td>
<td>• 6, 12, 18, &amp; 24 radial arm mazes</td>
<td>60 days</td>
<td>• ↑ Spatial memory (“chunking”)</td>
</tr>
<tr>
<td>Meck WH, Williams CL; <em>Neuroreport</em> 1997; 8: 3045-3051.</td>
<td>Pregnant Rats</td>
<td>E11-E18</td>
<td>• Peak interval timing procedure</td>
<td>4-6 months 24-26 months</td>
<td>• Suppl. ↑ Cognitive Function • Deficiency ↓ Attention</td>
</tr>
</tbody>
</table>

How Does Choline Impact The Developing Brain?

Cognitive Outcomes
- Attention
- Learning
- Memory

Neuronal Function
- Altered Acetylcholine Release from Cholinergic Neurons
- Altered Neural Pathways Involved In Memory

Brain Development
- Cellular Proliferation
- Neurogenesis
- Reduced Apoptosis
- Timing & Differentiation of Neuronal Progenitor Cells

DNA Methylation
- Altered Gene Expression
  - Synaptic Plasticity
  - Learning
  - Memory

Choline Intake During Pregnancy Is Associated With Childhood Cognition

**Study Goal**
To examine maternal 1\textsuperscript{st} and 2\textsuperscript{nd} 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 1\textsuperscript{st} and 2\textsuperscript{nd} trimesters of pregnancy
  - Offspring Visual Memory
    - Wide Range Assessment of Memory and Learning, 2\textsuperscript{nd} edition (WRAML2)

**Results & Conclusions**
Higher gestational choline intake was associated with better child visual memory at age 7

Low availability of choline \textit{in utero} disrupts development and function of the retina

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

**Defined Diet**
Modified AIN93G, AIN76A

- **Mating**
- **Pregnancy Day 11.5**

**Low Choline Diet**
(0 g choline / kg)

**Adequate Choline Diet**
(1.2 - 2g choline / kg)

- **≥1 Week**
- **11 days**
- **~6 days**

**Primary Endpoints:**
Retinal structure & function

How Does Maternal Choline Intake Impact Eye Development?

➢ 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

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.

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.

**Primary Endpoint:** Visual Attention Test

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

<table>
<thead>
<tr>
<th>Time</th>
<th>480 mg/day Choline (380 mg Dietary Choline + 100 mg VitaCholine)</th>
<th>930 mg/day Choline (380 mg Dietary Choline + 550 mg VitaCholine)</th>
</tr>
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<tbody>
<tr>
<td>~12 Weeks</td>
<td></td>
<td></td>
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<tr>
<td>4 Months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Months</td>
<td></td>
<td></td>
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<tr>
<td>10 Months</td>
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<tr>
<td>13 Months</td>
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</tr>
</tbody>
</table>

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.

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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

Mean Reaction Time Difference: 33.8 ms

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

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

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

480 mg/day Choline (380 mg Dietary Choline + 100 mg VitaCholine)

930 mg/day Choline (380 mg Dietary Choline + 550 mg VitaCholine)

~12 Weeks

4-13 Months

7 Years

Primary Endpoint:

N=20 children

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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)

Monitoring Interval (500, 1000, or 1500 ms)
Non-Signal or Signal Event (17, 29, or 50 ms)
Blank Screen (100 ms)
Response Cue (430 ms)
Response Window (<1500 ms)
Trial Feedback (500 ms)

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)
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

Source: Linus Pauling Institute, Oregon State University; [https://lpi.oregonstate.edu/mic/other-nutrients/choline](https://lpi.oregonstate.edu/mic/other-nutrients/choline); Alberts et al. (eds), Molecular Biology of the Cell, 1996.
Choline metabolome response to prenatal choline supplementation across pregnancy: A randomized controlled trial

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.

**N=30 pregnant women,** entering 2nd trimester (gestational week 12-16); age ≥ 21y;

**Primary Endpoints:**
- Markers of Choline Status

200 mg/day DHA + **25 mg/day VitaCholine**

(25 mg radiolabeled choline)

200 mg/day DHA + **550 mg/day VitaCholine**

(500 mg unlabeled + 50 mg radiolabeled choline)

~24-28 Weeks

Taesuwan 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

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(suppl): 1476S-1476S; RBC = Red Blood Cell
# Experts Recommend DHA During Pregnancy

<table>
<thead>
<tr>
<th>Expert Group</th>
<th>DHA Intake Recommendation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian National Health and Medical Research Council (NHMRC)</td>
<td>800 mg/day DHA + 100 mg/day EPA</td>
<td><a href="https://www.health.gov.au/resources/pregnancy-care-guidelines/part-c-lifestyle-considerations/nutrition-and-physical-activity#112-nutritional-supplements">https://www.health.gov.au/resources/pregnancy-care-guidelines/part-c-lifestyle-considerations/nutrition-and-physical-activity#112-nutritional-supplements</a></td>
</tr>
<tr>
<td>European Food Safety Authority (EFSA)</td>
<td>100-200 mg/day DHA</td>
<td>EFSA Journal 2010; 8(3): 1461</td>
</tr>
<tr>
<td>Global Organization for EPA and DHA Omega-3 (GOED)</td>
<td>300 mg/day DHA</td>
<td><a href="https://goedomega3.com/intake-recommendations">https://goedomega3.com/intake-recommendations</a></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Pregnancy Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low DHA</td>
<td></td>
</tr>
<tr>
<td>Medium DHA</td>
<td></td>
</tr>
<tr>
<td>High DHA</td>
<td></td>
</tr>
<tr>
<td>Low DHA</td>
<td>16 weeks</td>
</tr>
<tr>
<td>Medium DHA</td>
<td></td>
</tr>
<tr>
<td>High DHA</td>
<td></td>
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</table>

N=58 Puppies

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](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?

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.

**N=30 pregnant women,** entering 2nd trimester (gestational week 12-16); age ≥ 21y;

200 mg/day DHA + 25 mg/day *VitaCholine* (25 mg radiolabeled choline)

200 mg/day DHA + 550 mg/day *VitaCholine* (500 mg unlabeled + 50 mg radiolabeled choline)

~24-28 Weeks

**Primary Endpoints:**
- Markers of DHA Status
- Delivery: Placenta/Cord Blood

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
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.


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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.

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.”

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!**

- Suplemental **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!
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.”