The relation of human milk lutein, choline, and docosahexaenoic acid content to recognition memory abilities of 6-month-old breastfed infants

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Background

- Empirical evidence of a relation between nutrient intake and brain function has been elusive. The difficulty in documenting nutritional effects on brain could be because most research is focused on a single nutrient.
- *Early* choline status has been shown to be important for *later* adult memory in animal models. When *early* choline was tested in relation to *early* cognition, the results were mixed. Animal models show the integrity of the hippocampus is related to choline intake.
- Researchers report mixed results when supplementing infant diets with docosahexaenoic acid (DHA): fewer than 40% of trials find an effect on cognition in fullterm infants. DHA is high in the structures that underlie cognitive abilities, specifically the eye, the hippocampus, and the frontal brain areas. Importantly, DHA is co-localized with choline in the brain and liver.
- Lutein is deposited into the macula (which is the most immature structure of the eye at birth) concurrent with the development of visual acuity, which occurs across the first year of life.
- Nutrients do not exist in isolation nor is consumption limited to one type of food. Thus, nutrients may work synergistically in the brain. We tested the effects of choline, DHA, and lutein on recognition memory in 6-month-old infants using an electrophysiology paradigm known as event-related potentials (ERP).

Participants and Method

- Breastfeeding dyads were enrolled when the infants were 3-4 months old. Exclusive breastfeeding was defined as no more than 10% supplemented. All but two of the infants were 100% breastfed; the two met criteria. Milk samples were obtained by expressing the milk from one breast and providing aliquots that contained a mix of fore- and hindmilk.
- In the NORC, breastmilk samples were analyzed for fatty acid content. Lipids were extracted and saponified, with resulting fatty acids trans-methylated to methyl esters which were analyzed using capillary gas chromatography.
- Free choline and betaine were assayed in the Innis lab as detailed in Innis and Hassman, and lutein content was determined using a liquid:liquid extraction method as in Yuhas et al.
- At 6 months of age, a random selection of participants were invited back for an electrophysiology session. Infants were fitted with a 128-channel HydroCel Geodesic Sensor Net (Electrical Geodesics, Inc). The infants were habituated to a picture of a wooden toy. Then, they participated in a 70-30 oddball paradigm that included a random presentation of 70 of their familiar picture and 30 trial-unique pictures.
- Data were manually examined for artifacts, and video was viewed frame by frame to identify segments in which the infants were looking at the stimuli. Only artifact-free trials in which infants were attending to the stimuli were included. Participants who provided at least 10 trials (m = 18, range 10-40) in each condition were included in the analyses (n=55).
- Trials were averaged across condition; novel were subtracted from familiar data. Resulting data were averaged in sensor groupings as shown. Latency to peak amplitude, peak amplitude, and mean amplitude were analyzed in full model multivariate regressions in SAS 9.2.
- Stepwise regressions were undertaken to build models. Significant interactions were followed up with simple slopes analyses.

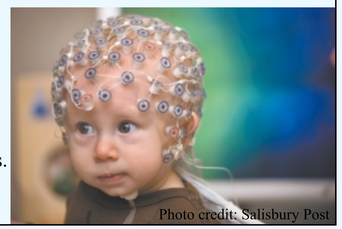
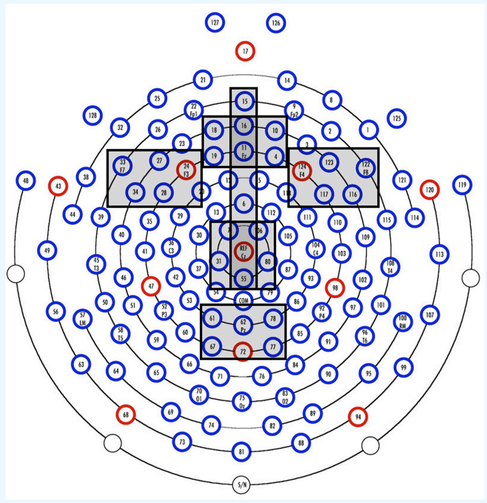


Photo credit: Salisbury Post

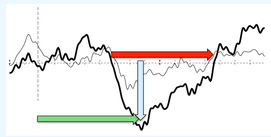
Milk Analyses					
	n	Min	Max	Mean	SE
Infant age at sample	60	3.0	5.6	3.6	-
DHA (g/100g)	61	0.08	1.0	0.26	0.02
Free choline (umol/L)	60	23.0	326.9	158.4	8.9
Lutein (mcg/L)	62	0.0	52.6	18.4	1.9



128-Channel HydroCel Geodesic Sensor Map (Electrical Geodesics, Inc).

Sensors were grouped as follows:
FrontalZ (4, 10, 11, 16, 18, 19)
FrontalL (24, 27, 28, 34, 33)
FrontalR (116, 117, 122, 123, 124)
CentralZ (7, 31, 55, 80, 106, REF)
ParietalZ (61, 62, 67, 72, 77, 78)
Midline (6, 11, 15, 16, 55, REF)

Familiar – Novel = Outcome data
Measurements –
Mean amplitude
Peak amplitude
Latency to peak
Window – 200 – 900 ms after stim



Results of Full & Reduced Model Analyses

- Full model:

FtlZ FrtL FrtR CntlS PrtlZ Midl = Intercept + DHA + Cho + Lut + ChoXLut + ChoXDHA + Err

  - No variable predicted mean or peak amplitude at any sensor grouping.
  - Latency to peak amplitude was predicted by choline at frontal right leads ($p=0.05$) and by choline ($p<0.01$) and lutein by choline interaction ($p<0.05$) at the central leads.

- Reduced Choline model predicting latency to peak amplitude:

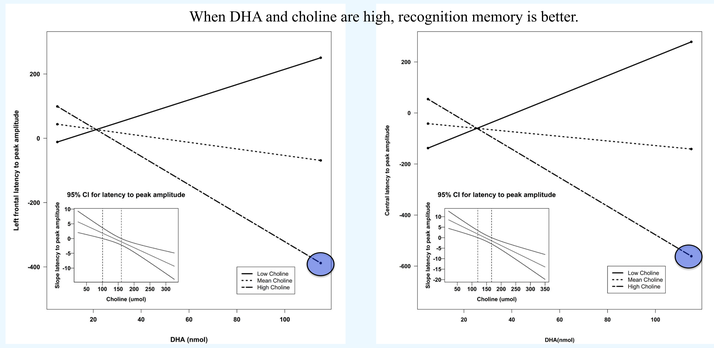
Frontal Left
-117.69 + 6.81 DHA + 1.05 Choline – 0.05 DHA X Choline Model $p < 0.05$ R-sq = 0.18
Central Z
-312.00 + 9.89 DHA + 1.73 Choline – 0.07 DHA X Choline Model $p < 0.01$ R-sq = 0.21

- Reduced Lutein model predicting latency to peak amplitude:

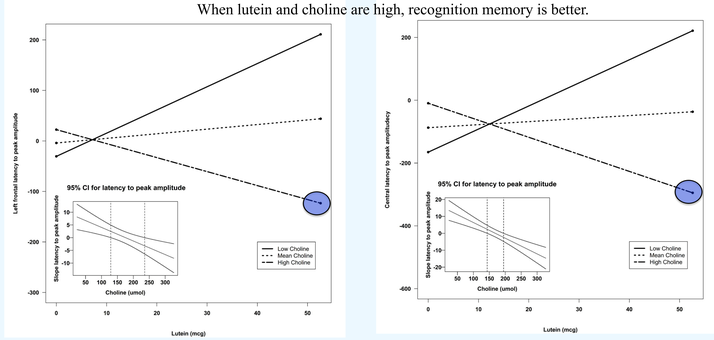
Frontal Left
-64.97 + 9.38 Lutein + 0.38 Choline – 0.05 Lut X Choline Model $p < 0.10$ R-sq = 0.13
Central Z
-266.55 + 15.66 Lutein + 1.13 Choline – 0.09 Lut X Choline Model $p < 0.01$ R-sq = 0.24

Simple Slopes Models: Latency at Frontal & Central Sensors

When DHA and choline are high, recognition memory is better.



When lutein and choline are high, recognition memory is better.



Discussion

- Recognition memory abilities are supported by high DHA and choline as well as high lutein and choline. Importantly, there was no significant relation between DHA or lutein and the cognitive measures. Thus, the nutrients are needed together to support memory.
- The relation was found only in latency to peak amplitude. There was no significant association with mean amplitude or peak amplitude. Moreover, the significant relations were confined to the central and frontal areas of the scalp.
- Latency to peak amplitude is interpreted as a measure of sustained processing. When the difference between activity in response to novel and familiar stimuli is large, we infer recognition memory. If a stimulus is remembered, it does not require processing.
- Phosphatidylcholine (PC) serves an active role in the hepatic export of DHA to the brain. It is possible that higher choline allows for a higher rate of DHA transfer.
- To our knowledge, this is the first evidence of synergy of human milk nutrients in support of cognitive abilities, in the absence of single nutrient support.

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