

# DIETARY INTAKE PATTERN OF CHOLINE BY PREGNANT WOMEN AT THIRD TRIMESTER

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**Abstract:** A higher maternal choline intake during the third trimester of human pregnancy may counter some of the adverse effects of maternal prenatal stress, behavioral, neuroendocrine problem and metabolic development in the offspring. One hundred and three pregnant women visited antenatal care clinic of Azimpur Maternity Hospital of Dhaka was selected for the present study. The 24 hour dietary recall method was used to find out intake pattern of choline. Mean consumption of choline was less than the recommended choline intake  $193.96 \pm 100.5$ , this was 42.72% of total RDA value. The results of this study indicate that present choline status among pregnant women is not satisfactory. Curbing the deficiency epidemic among pregnant women of Bangladesh, increased education, including recommendations to consume more choline-rich foods, is needed to improve choline intakes for optimal health.

**Key words:** Choline; Pregnancy; Fetal development; Cognition; Brain function

## Introduction

Choline, an essential nutrient<sup>1,2,3,4,5,6,7</sup> plays a significant role in human pregnancy to reduce the negative effect of mother's stress<sup>8</sup> on child health<sup>9</sup> promoting fetal growth<sup>10-12</sup>, proper brain<sup>13-19</sup> and memory function<sup>20-26</sup> and learning capabilities<sup>27-31</sup> with a guard for future health of child. During pregnancy, women face stress having poverty, relationship insecurity, family crisis, unhealthy lifestyle, occupational disturbance, lack of knowledge and therefore their stress level directly affects their child health regarding impaired brain development, low memory and learning function, lowered resistance to infection, poor cognitive development and decreased work productivity and most probably responsible for future risk of chronic diseases including hypertension, diabetes and mental disorders. Several study findings raise the exciting possibility that a higher maternal choline intake may counter some of the adverse effects<sup>31-33</sup> of prenatal stress on behavioral<sup>34-36</sup>, neuroendocrine and metabolic development<sup>37-38</sup> in the offspring. The higher intake of choline contributed to a more stable HPA axis<sup>8</sup>, which in turn meant lower cortisol levels in the fetus. The changes in fetal genetic expression will likely continue into adulthood, where they play a role in stress related disease prevention<sup>39</sup>. Dietary intake of choline by the pregnant mother and later by the infant directly affects brain development and results in permanent changes in brain and memory function.<sup>40,41,42</sup> Memory can be permanently enhanced exposing to choline during the latter part of gestation.<sup>33</sup> Another study showed that when rat pups received choline supplements, their brain function changed, resulting in the lifelong memory enhancement.<sup>34</sup> A retrospective study showed that increased dietary intake of choline early in life improves performance of adult rats on memory tasks and prevents their age-related memory decline.<sup>45</sup> Choline supplementation during gestation in rats leads to augmentation of spatial memory in adulthood<sup>46</sup>, improves brain



function of the animal's offspring and enhances two proteins involved in learning and memory, according to a new study. Maternal choline appears to decrease the risk of neural tube defect.<sup>47,48,49</sup> Study showed that a deficiency of choline substantially impaired the body's ability to regulate homocysteine levels.<sup>50</sup> Excessive homocysteine is apparently linked with increased risks for birth defects, cardiovascular disease<sup>51</sup>, cancer, type 2 diabetes, hypertension, depression and more. Higher intakes of dietary choline is related to lower homocysteine concentrations.<sup>52,53</sup> Foods rich in choline may help reduce the risk of inflammation associated with chronic diseases such as cardiovascular disease, bone loss, dementia and Alzheimer's disease.<sup>54</sup> A study funded by the National Institutes of Health concluded that dietary choline in pregnancy is associated with a 24 percent reduced risk of breast cancer in female offspring.<sup>55,56</sup> Tumor growth rate was inversely related to choline content in the prenatal diet, resulting in 50% longer survival. Choline deficient diet during pregnancy adversely affects immunity, growth, cognitive development and causes apathy, which affects school performance and social development and is also responsible for increased risk of complications during delivery, including prolonged labour, preterm delivery, preeclampsia, prematurity, neural tube defects, very low birth weight<sup>57</sup> and maternal and neonatal death.

Most of the Bangladeshi pregnant women don't meet their recommended level of micronutrients including choline which can be effective during pregnancy fighting against different physical and mental complications. The main objective of this study was determining the present status of choline intake pattern by Bangladeshi pregnant women at their third trimester of pregnancy.

## Methodology

A cross sectional study was carried out from 4<sup>th</sup> May to 6<sup>th</sup> June, 2013 at Azimpur maternity hospital, Dhaka, Bangladesh. A total number of one hundred and three pregnant women at their third trimester of pregnancy without any complication were selected by systemic random system from the maternity hospital. Informed consents were taken to cooperate with interviewers getting the interview. A semi-structured pre-tested questionnaire was developed considering their age, occupation, stage of pregnancy, weight, height and educational level. A 24 hour dietary recall form was introduced to get the information regarding their dietary intake pattern for the last 24 hours representing their daily dietary schedule. Data was collected through each interview session. Intakes of choline were calculated from food-composition tables multiplying the frequency of consumption of each food item by its choline content and summing the nutrient contributions of all foods. All of the collected data were analyzed by using SPSS v-15.0 program. Descriptive statistics including mean, standard deviation, and frequency were obtained.

## Result

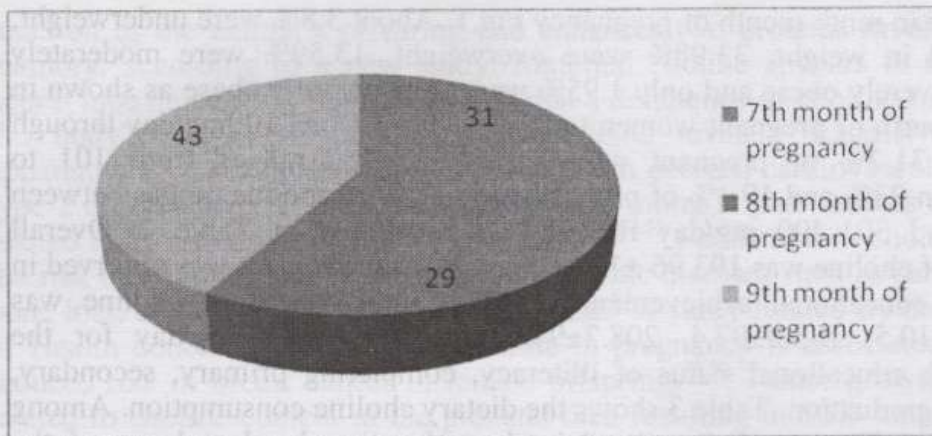
Among 103 pregnant women in the Azimpur Maternity Hospital, 69.9% were in the age group of 21-25 years, 27.2% were in the age group of 26-30 years and 2.9 were 3% >31 years. Majority of the pregnant women of Dhaka, Bangladesh were in 21-25 years. About 6.8% of pregnant women were illiterate, 10.7% of pregnant women had primary, 56.3% secondary, 11.7% under graduate while 14.5% were graduate or above. Majority of the pregnant women completed their secondary educational level, 87.4% pregnant women was house wife and 12.6% were service holder. About 30.1% of pregnant women were in seventh month of pregnancy, 28.16% were in eighth month of pregnancy and 41.74% of



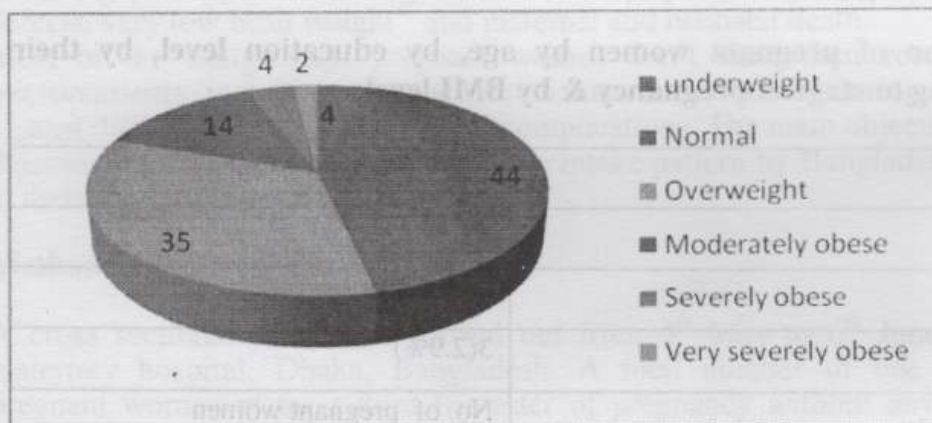
pregnant women were in ninth month of pregnancy Fig.1. About 3.8% were underweight, 42.72% were normal in weight, 33.98% were overweight, 13.59% were moderately obese, 3.88% were severely obese and only 1.95% were very severely obese as shown in Table 1. Fig 2 One fourth of pregnant women took choline less than 100mg/day through regular diet. While 31.2% of pregnant women took choline ranges from 101 to 200mg/day. More than 23% and 19.4% of pregnant women took choline ranges between 201 and 300mg/day and 301-400 mg/day respectively as shown in Table 2. Overall average intake level of choline was  $193.96 \pm 100.5$ . Parallel improvement was observed in choline intake with educational achievement. Average intake level of choline was  $147.7 \pm 83.3$ ,  $157.8 \pm 110.5$ ,  $185.9 \pm 97.4$ ,  $208.2 \pm 94.2$  and  $231.4 \pm 94.9$  mg/day for the pregnant women with educational status of illiteracy, completing primary, secondary, under graduation and graduation. Table 3 shows the dietary choline consumption. Among the considered factors affecting choline level intake, education level and age of the pregnant women's have substantial effect. Both of the factors are positively correlated with choline level intake though the amount of correlation is weak but surely they have a noticeable influence on the intake of choline level as shown in Table 4.

**Table 1: Distribution of pregnant women by age, by education level, by their occupation, according to stage of pregnancy & by BMI level.**

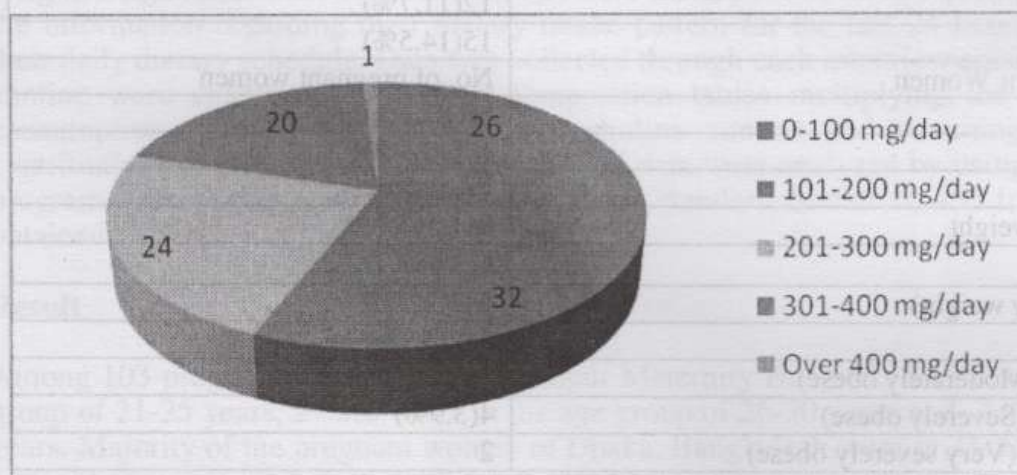
Age in year	No of pregnant women
<20	24(23.3%)
26-30	28(27.2%)
> 31	3(2.9%)
Level of education	No. of pregnant women
Illiterate	7(6.8%)
Primary	11(10.7%)
Secondary	58(56.3%)
Under Graduate	12(11.7%)
≥ Graduate	15(14.5%)
Type of Pregnant Women	No. of pregnant women
House wife	90(87.4%)
Service holder	13(12.6%)
BMI status	Frequencies
severely underweight	0
Underweight	4(3.88%)
Normal (healthy weight)	44(42.72%)
Overweight	35(33.98%)
Obese Class I (Moderately obese)	14(14%)
Obese Class II (Severely obese)	4(3.9%)
Obese Class III (Very severely obese)	2



**Fig-1: Distribution of total number (n=103) of pregnant women according to Stage of pregnancy**



**Fig-2: Total number (n=103) of pregnant women by their nutritional status using BMI level**



**Fig 3: Distribution of total numbers (n=103) of pregnant women by daily intake level of choline (mg/day)**



**Table 3: Gradual improvement of average choline intake per day with educational status**

Educational level	Average choline intake/day(mg)
Illiterate	147.7±83.3
Primary	157.8±110.5
Secondary	185.9±97.4
Under Graduate	208.2±94.2
≥ Graduate	231.4±94.9

**Table 4: Correlation Analysis:**

Correlations			
	Choline Level	Education Level	Age
Choline Level	1	0.186	0.166
Education Level	0.186	1	-0.008
Age	0.166	-0.008	1

## Discussion

In this study, an assessment of dietary choline intake was made using a 24 hours dietary recall system to record the food items taken by pregnant women for last 24 hours. In this analyses, it has been found that choline status of Bangladeshi pregnant women is very disappointing delivered less daily choline than the adequate intake quoted by the Institute of Medicine of the National Academy of Sciences, USA (450 mg/day). The study suggests that pregnant women in Bangladesh are eating foods that may not be delivering adequate amounts of choline.

In new Zealand, a study<sup>59</sup> on 125 pregnant women showed that mean (SD) daily intake of choline was 316 (66) mg, where this study reported the mean (SD) daily intake of choline with 193.96 ±100.5 mg by Bangladeshi pregnant women. Another study showed that mean intake estimates for choline among 188,147 participants (aged 45-75) was 304 mg/d in women<sup>60</sup> where the mean intake of choline by Bangladeshi pregnant women was determined as 193.96 ±100.5 mg/d. Another study showed the mean intake of choline by common food sources among Taiwanese female population<sup>61</sup> estimated as 265±9 mg/d, where Bangladeshi pregnant women took only 193.96 ±100.5mg/d on an average. In one study conducted in Jamaica also showed the poor choline status among pregnant women with 278.5 mg/day<sup>62</sup> which was higher than the Bangladeshi scenario.

An adequate intake level of 450 mg/day was established<sup>63</sup> for women during pregnancy wherease this study found that Bangladeshi pregnant women took about 189.5mg/day providing only 42.72% of RDA value. Although the implications of inadequate choline in



the diet have not been fully examined in humans, several animal studies show that in terms of memory development, the role of dietary choline during pregnancy is significant.<sup>64-68</sup> Choline in the mother's diet led to a more stable HPA axis and consequently less cortisol in the fetus. This study evaluated that choline status in Bangladesh is not also satisfactory. Poverty and lack of knowledge regarding the importance of choline among both pregnant women and health care professionals promote less choline supplementation during pregnancy. The ideal age of pregnancy is 19-30 years. It was found that among the pregnant women of Dhaka, Bangladesh, majority were in 20-25 years. Maternal education level has a significant effect on choline status in pregnancy. Present study showed that, a tendency towards an increase consumption of choline in pregnancy with an increase in the level of mother education. This may due to better awareness of health.. In this study, most of the pregnant women were completed their secondary level of education. About 43% of pregnant women had normal weight, 13.59%, 3.88% and 1.95% of pregnant women had moderately, over weight and obese. Parallel improvement was observed in choline intake with educational level. Average intake level of choline was  $147.7 \pm 83.3$ ,  $157.8 \pm 110.5$ ,  $185.9 \pm 97.4$ ,  $208.2 \pm 94.2$  and  $231.4 \pm 94.9$  mg/day for the pregnant women with educational status of illiteracy, completing primary, secondary, under graduation and graduation. The overall survey result also shows that, most of the Bangladeshi pregnant women don't take the recommended level of choline which is 450 mg/day. This is the first study evaluating dietary intake pattern of choline in the Bangladeshi population and suggests a need to assess further whether diets of this population ensure adequate plasma choline supplementation during pregnancy.

### Conclusion

Choline is considered as mostly neglected micronutrient by both pregnant women and health care professionals having poor knowledge regarding its importance and therefore missed out in maternal diet causing several health complications beyond consideration. The results of this study may be an indication that the choline included in the diet of pregnant women in Bangladesh may not be adequate to meet both the needs of the mother and fetus that further studies are warranted to determine clinical implications. It is hoped that the study would be useful in understanding the present choline status among Bangladeshi pregnant women aiming to improve using strategic nutritional intervention by both government and public stakeholders.

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## References

1. Mehedint MG, Craciunescu CN, Zeisel SH. Maternal dietary choline deficiency alters angiogenesis in fetal mouse hippocampus. *Proc Natl Acad Sci U S A*. 2010 Jul 20;107(29):12834-9. doi: 10.1073/pnas.0914328107. Epub 2010 Jul 12.
2. Albright CD, Tsai AY, Friedrich CB, Mar MH, Zeisel SH. Choline availability alters embryonic development of the hippocampus and septum in the rat. *Brain Res*. 1999;113:13-20.
3. Craciunescu CN, Albright CD, Mar MH, Song J, Zeisel SH. Choline availability during embryonic development alters progenitor cell mitosis in developing mouse hippocampus. *J. Nutr*. 2003;133:3614-18.
4. Steven H. Zeisel, MD, PhD. Nutritional Importance of Choline for Brain Development. *J Am Coll Nutr*, December 2004; 23(6): 621S-626S
5. Michel V, Bakovic M. Editorial: choline and brain function. *Cent Nerv Syst Agents Med Chem*. 2012 Jun;12(2):69.
6. Zeisel SH. The supply of choline is important for fetal progenitor cells. *Semin Cell Dev Biol*. 2011 Aug;22(6):624-8. doi: 10.1016/j.semcd.2011.06.002. Epub 2011 Jun 12.
7. C D Albright, A Y Tsai, C B Friedrich, M H Mar, S H Zeisel . Choline availability alters embryonic development of the hippocampus and septum in the rat. *Brain Res Dev Brain Res*. 1999 Mar 12;113(1-2):13-20.
8. Steven H. Zeisel, MD, PhD and Mihai D. Niculescu, MD, PhD. Perinatal Choline Influences Brain Structure and Function. *Nutr Rev*. 2006 April; 64(4):197-203
9. Wong-Goodrich SJ, Glenn MJ, Mellott TJ, Blusztajn JK, Meck WH, Williams CL. [http://www.ncbi.nlm.nih.gov/pubmed?term=Glenn%20MJ%5BAuthor%5D&cauthor=true&cauthor\\_uid=18778697](http://www.ncbi.nlm.nih.gov/pubmed?term=Glenn%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=18778697)Spatial memory and hippocampal plasticity are differentially sensitive to the availability of choline in adulthood as a function of choline supply in utero. *Brain Res*. 2008 Oct 27;1237:153-66. doi: 10.1016/j.brainres.2008.08.074. Epub 2008 Sep 4.
10. Niculescu MD, Craciunescu CN, Zeisel SH. Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. *FASEB J*. 2006 Jan;20(1):43-9.
11. Mihai G. Mehedint, Corneliu N. Craciunescu, and Steven H. Zeisel. Maternal dietary choline deficiency alters angiogenesis in fetal mouse hippocampus. *Proc. Natl. Acad. Sci. USA* (2010)107 (29):12834-12839 <http://www.pnas.org/content/107/29/12834.full> - corresp-1
12. Mihai G. Mehedint, Mihai D. Niculescu, Corneliu N. Craciunescu and Steven H. Zeisel . Choline deficiency alters global histone methylation and epigenetic marking at the *Rel* site of the calbindin 1 gene *FASEB J*. (2010)24 (1):184-195
13. Steven H Zeisel <http://ajcn.nutrition.org/content/89/2/673S.abstract> - fn-1. Importance of methyl donors during reproduction *Am J Clin Nutr*(2009)89 (2):673S-677S
14. Vesela P, Kovacheva, Tiffany J. Mellott, Jessica M. Davison, Nicholas Wagner, Ignacio Lopez-Coviella, Aletta C. Schnitzler. Gestational Choline Deficiency Causes Global and *Igf2* Gene DNA Hypermethylation by Up-regulation of *Dnmt1* Expression *J Biol Chem*(2007)282 (43):31777-31788
15. Tiffany J. Mellott\*, Maximillian T. Follettie‡, Veronica Diesl‡, Andrew A. Hill‡, Ignacio Lopez-Coviella\*, †Jan Krzysztof Blusztajn\* Prenatal choline availability modulates hippocampal and cerebral cortical gene expression. *The FASEB Journal*. 2007, 21( 7):1311-1323
16. Boeke CE, Gillman MW, Hughes MD, Rifas-Shiman SL, Villamor E, Oken E. [http://www.ncbi.nlm.nih.gov/pubmed?term=Gillman%20MW%5BAuthor%5D&cauthor=true&cauthor\\_uid=23425631](http://www.ncbi.nlm.nih.gov/pubmed?term=Gillman%20MW%5BAuthor%5D&cauthor=true&cauthor_uid=23425631)Choline intake during pregnancy and child cognition at age 7 years. *Am J Epidemiol*. 2013 Jun 15;177(12):1338-47. doi: 10.1093/aje/kws395. Epub 2013 Feb 20.



17. Brian T. F. Wu, Roger A. Dyer, D. Janette King, Kelly J. Richardson, Sheila M. Innis. Early second trimester maternal plasma choline and betaine are related to measures of early cognitive development in term infants. *PLoS One*. 2012;7(8):e43448. doi: 10.1371/journal.pone.0043448. Epub 2012 Aug 20
18. Zeisel SH. Choline: needed for normal development of memory. *J Am Coll Nutr*. 2000 Oct;19(5 Suppl):528S-531S.
19. Blusztajn JK, Mellott TJ. Neuroprotective actions of perinatal choline nutrition. *Clin Chem Lab Med*. 2013 Mar 1;51(3):591-9. doi: 10.1515/cclm-2012-0635.
20. Hind Beydoun, Audrey F. Saftlas. Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. *Paediatric and Perinatal Epidemiology*. Sep 2008; 22(5): 438–466
21. Marasco V, Robinson J, Herzyk P, Spencer KA. Pre- and post-natal stress in context: effects on the stress physiology in a precocial bird. *J Exp Biol*. 2012; 15;215(Pt 22):3955-64. doi: 10.1242/jeb.071423. Epub 2012 Aug 16.
22. Catalani A, Alemà GS, Cinque C, Zuena AR, Casolini P. Maternal corticosterone effects on hypothalamus-pituitary-adrenal axis regulation and behavior of the offspring in rodents. *Neurosci Biobehav Rev*. 2011 Jun; 35(7):1502-17. doi: 10.1016/j.neubiorev.2010.10.017. Epub 2010 Nov 4.
23. Macrì S, Zoratto F, Laviola G. Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer. *Neurosci Biobehav Rev*. 2011 Jun;35(7):1534-43. doi: 10.1016/j.neubiorev.2010.12.014. Epub 2011 Jan 7.
24. Mairesse J, Lesage J, Breton C, Bréant B, Hahn T, Darnaudéry M, et al. Maternal stress alters endocrine function of the feto-placental unit in rats. *Am J Physiol Endocrinol Metab*. 2007 Jun;292(6):E1526-33. Epub 2007 Jan 30.
25. Rangon CM, Fortes S, Lelièvre V, Leroux P, Plaisant F, Joubert C, Lanfumey L, et al. Chronic mild stress during gestation worsens neonatal brain lesions in mice. *J Neurosci*. 2007 Jul 11;27(28):7532-40.
26. Hirst JJ, Walker DW, Yawno T, Palliser HK. Stress in pregnancy: a role for neuroactive steroids in protecting the fetal and neonatal brain. *Dev Neurosci*. 2009;31(5):363-77. doi: 10.1159/000232555. Epub 2009 Aug 15.
27. Son GH, Geum D, Chung S, Kim EJ, Jo JH, Kim CM, et al. Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006 Mar 22, 26(12): 3309-18
28. Wilkinson J. High maternal choline intake may prevent the development of stress-related disorders through epigenetic mechanisms. *Epigenomics*. 2012 Oct;4(5):479-80.
29. Steven H. Zeisel. THE FETAL ORIGINS OF MEMORY: THE ROLE OF DIETARY CHOLINE IN OPTIMAL BRAIN DEVELOPMENT. *J Pediatr*. 2006 November; 149(5 Suppl): S131–S136.
30. Zeisel SH. Choline: essential for brain development and function. *Adv Pediatr*. 1997;44:263-95.
31. Craciunescu CN, Albright CD, Mar MH, Song J, Zeisel SH. Choline availability during embryonic development alters progenitor cell mitosis in developing mouse hippocampus. *J. Nutr*. 2003;133:3614–18.
32. Steven H. Zeisel, MD, PhD and Mihai D. Niculescu, MD, PhD. Perinatal Choline Influences Brain Structure and Function. *Nutr Rev*. 2006 April; 64(4):197-203
33. Zeisel SH. Choline: needed for normal development of memory. *J Am Coll Nutr*. 2000 Oct;19(5 Suppl):528S-531S.
34. Melissa J. Glenn, Erin M. Gibson, [...], and Christina L. Williams. Prenatal choline availability modulates hippocampal neurogenesis and neurogenic responses to enriching experiences in adult female rats. *Eur J neurosci*. 2007, 25(8):2473-2482.



35. Pyapali, Gowri K., Dennis A. Turner, Christina L. Williams, Warren H. Meck, and H. Scott Swartzwelder. Prenatal dietary choline supplementation decreases the threshold for induction of long-term potentiation in young adult rats. *J. Neurophysiol.* 1998, 79:1790–1796.
36. Shaw G, Carmichael S, Yang W, Selvin S, Schaffer D. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. *Am J Epidemiol.* 2004; 160:102–109.
37. Rees W, Wilson F, Maloney C. Sulfur amino acid metabolism in pregnancy: the impact of methionine in the maternal diet. *J Nutr.* 2006; 136:1701S–1705S.
38. Gary M. Shaw<sup>1</sup>, Suzan L. Carmichael<sup>1</sup>, Wei Yang<sup>1</sup>, Steve Selvin<sup>2</sup> and Donna M. Schaffer. Periconceptional Dietary Intake of Choline and Betaine and Neural Tube Defects in Offspring *Am. J. Epidemiol.* (2004) 160 (2): 102-109. doi: 10.1093/aje/kwh187
39. Anne M Molloy <http://ajcn.nutrition.org/content/82/4/836.full> - aff-1, James L Mills <http://ajcn.nutrition.org/content/82/4/836.full> - aff-1, Christopher Cox <http://ajcn.nutrition.org/content/82/4/836.full> - aff-1, Sean F Daly <http://ajcn.nutrition.org/content/82/4/836.full> - aff-1, Mary Conley <http://ajcn.nutrition.org/content/82/4/836.full> - aff-1, Lawrence C Brody <http://ajcn.nutrition.org/content/82/4/836.full> - aff-1, et al. Choline and homocysteine interrelations in umbilical cord and maternal plasma at delivery. *Am J Clin Nutr* October 2005 vol. 82 no. 4 836-842
40. Jessica Chan, Liyuan Deng, Leonie G Mikael, Jian Yan, Laura Pickell, Qing Wu, et al. Low dietary choline and low dietary riboflavin during pregnancy influence reproductive outcomes and heart development in mice *Am J Clin Nutr* (2010) 91 (4):1035-1043
41. Eunyoung Cho <http://ajcn.nutrition.org/content/83/4/905.abstract> - aff-1, Steven H Zeisel <http://ajcn.nutrition.org/content/83/4/905.abstract> - aff-1, Paul Jacques <http://ajcn.nutrition.org/content/83/4/905.abstract> - aff-1, Jacob Selhub <http://ajcn.nutrition.org/content/83/4/905.abstract> - aff-1, Lauren Dougherty <http://ajcn.nutrition.org/content/83/4/905.abstract> - aff-1, Graham A Colditz <http://ajcn.nutrition.org/content/83/4/905.abstract> - aff-1, et al. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *Am J Clin Nutr* (2006) 83 (4):905-911
42. Paraskevi Detopoulou <http://ajcn.nutrition.org/content/87/2/424.abstract> - aff-1, Demosthenes B Panagiotakos <http://ajcn.nutrition.org/content/87/2/424.abstract> - aff-1, Smaragdi Antonopoulou <http://ajcn.nutrition.org/content/87/2/424.abstract> - aff-1, Christos Pitsavos <http://ajcn.nutrition.org/content/87/2/424.abstract> - aff-1, Christodoulos Stefanadis. Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study *Am J Clin Nutr* (2008) 87 (2):424-430
43. Cho E, et al. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *AJCN.* 2006; 83:905-11.
44. Xu X, et al. Choline metabolism and risk of breast cancer in population-based study. *FASEB J.* 2008; 22:1-8. vi Cho E, et al. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *AJCN.* 2006; 83:905-11.
45. Vesela P, Kovacheva, Jessica M. Davison, Tiffany J. Mellott, Adrienne E. Rogers, Shi Yang, Michael J. O'Brien, et al. Raising gestational choline intake alters gene expression in DMBA-evoked mammary tumors and prolongs survival *FASEB J.* (2009) 23 (4):1054-1063
46. Stein Emil Vollset, Helga Refsum, Lorentz M Irgens, Barbro Mork Emblem, Aage Tverdal, Håkon K Gjessing, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. *Am J Clin Nutr.* 2000; 71:962-968.
47. Allen MC. Neurodevelopmental outcomes of preterm infants. *Curr Opin Neurol.* 2008; 21:123–8.



48. Mygind VL, Evans SE, Peddie MC, Miller JC, Houghton LA. Estimation of usual intake and food sources of choline and betaine in New Zealand reproductive age women. *Asia Pac J Clin Nutr.* 2013;22(2):319-24. doi: 10.6133/apjcn.2013.22.2.19.
49. Yonemori KM, Lim U, Koga KR, Wilkens LR, Au D, Boushey CJ, Le Marchand L, Kolonel LN, Murphy SP. Dietary choline and betaine intakes vary in an adult multiethnic population. *J Nutr.* 2013 Jun;143(6):894-9. doi: 10.3945/jn.112.171132. Epub 2013 Apr 24.
50. Da-MingChu, Mark L Wahlqvist, HsingYi Chang, NaiHuaYeh, MeeiShyuan Lee. Choline and betaine food sources and intakes in Taiwanese. *Asia Pac J Clin Nutr* 2012;21 (4):547-557
51. M Gossell-Williams H Fletcher, N McFarlane-Anderson, A Jacob, J Patel, and S Zeisel. Dietary Intake of Choline and Plasma Choline Concentrations in Pregnant Women in Jamaica. *West Indian Med J.* 2005 December; 54(6): 355-359.
52. Caudill MA. Pre- and postnatal health: evidence of increased choline needs. *J Am Diet Assoc.* 2010 Aug;110(8):1198-206. doi: 10.1016/j.jada.2010.05.009.
53. Albright CD, Friedrich CB, Brown EC, Mar MH, Zeisel SH. Maternal dietary choline availability alters mitosis, apoptosis and the localization of TOAD-64 protein in the developing fetal rat septum. *Brain Res Dev Brain Res.* 1999;115:123-9. [PubMed]
54. Albright CD, Tsai AY, Friedrich CB, Mar MH, Zeisel SH. Choline availability alters embryonic development of the hippocampus and septum in the rat. *Brain Res Dev Brain Res.* 1999;113:13-20. [PubMed]
55. Fisher MC, Zeisel SH, Mar MH, Sadler TW. Perturbations in choline metabolism cause neural tube defects in mouse embryos in vitro. *FASEB J.* 2002;16:619-21.