Published in final edited form as:
Metabolism. 2008 October ; 57(Suppl 2): S6–10. doi:10.1016/j.metabol.2008.07.007.

Synapse Formation and Cognitive Brain Development: effect of docosahexaenoic (DHA) and other dietary constituents

R. J. Wurtman
Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge MA, 02139, USA

Abstract

The brain is unusual among organs in that the rates of many of its characteristic enzymatic reactions are controlled by the local concentrations of their substrates, which also happen to be nutrients that cross the blood-brain barrier. Thus, for example, brain levels of tryptophan, tyrosine, or choline can control the rates at which neurons synthesize serotonin, dopamine, or acetylcholine, respectively. The rates at which brain cells produce membrane phospholipids like phosphatidylcholine (PC) are also under such control, both in adult animals and, especially, during early development. If pregnant rats are fed the three dietary constituents needed for PC synthesis - docosahexaenoic acid (DHA), uridine, and choline - starting 10 days before parturition and continuing for 20 days during nursing, brain levels of PC and of the other membrane phosphatides (per cell or per mg protein) are increased by 50% or more. In adult animals this treatment is also known to increase synaptic proteins (e.g. synapsin-l; syntaxin-3; GluR-l; PSD-95) but not ubiquitous proteins like beta-tubulin, and to increase (by 30% or more) the number of dendritic spines on hippocampal neurons. DHA currently is widely used, in human infants, to diminish the negative effects of prematurity on cognitive development. Moreover, DHA, uridine (as UMP), and choline are all found in mother's milk, and included in most infant formulas. It is proposed that these substances are part of a regulatory mechanism through which plasma composition influences brain development.

Keywords

omega-3 fatty acids; phosphatidylcholine; uridine; arachidonic acid; phosphatidylcholine

Introduction

Omega-3 Fatty Acids and the Brain

The phospholipids in brain membranes contain many different fatty acids (c.f. ref. 1). However one such compound, the omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA), is both uniquely abundant among them (2) and particularly important in the development and maintenance of brain mechanisms underlying cognitive functions (3). Thus, cognitive development among breast-fed full-term infants, or in full-term or preterm infants given supplemental DHA, is described as being superior to that in infants consuming formula diets that lack DHA (4); and the consumption, by term infants of a formula supplemented with DHA

Corresponding Author: R. J. Wurtman, Address: MIT, 43 Vassar St., 46-5023, Cambridge MA, 02139, USA, Phone: +1-617-253-6731, Fax: +1-617-253-6882, E-mail address: dick@mit.edu.

(plus the omega-6 fatty acid arachidonic acid [AA]) during the first 17 weeks of life increased test scores on the Mental Development Index, assessed a year or more later (5). At the other end of life, DHA levels in plasma phosphatidylcholine (PC) are inversely correlated with the risk of developing dementia, as shown in aged participants (average: 76 years) enrolled in the Framingham Heart Study and followed for 9 years (6), and high intakes of fish or of DHA have been described by most investigators as protective against age-related cognitive decline and the risk of developing Alzheimer's Disease (7,8,9). DHA administration has also been found to produce dose-related improvements in cognitive functions in various experimental animals (c.f. ref. 10).

A number of hypotheses have been proposed to explain the beneficial effects of DHA consumption on brain functions and, particularly, cognition. These include, among others, changing the “fluidity” of neuronal membranes (11), and thereby alternating the activities of receptors, ion channels, G-proteins, and other proteins embedded in the membranes; being transformed to active metabolites (12) like “neuroprotectin D1” (10,17S-docosatriene) which reportedly suppresses A-beta-42 neurotoxicity (13) or to the prostaglandin-like F4-neuroprostanes (14); promoting neurogenesis by causing the differentiation of neuronal stem cells (15); activating syntaxin-3, a synaptic membrane protein that promotes neurite outgrowth (16); decreasing the AA content of brain phospholipids (17); or forming DHA-rich diacylglycerols which are preferentially utilized for synthesizing membrane phosphatides via the Kennedy Cycle (18).

Oral DHA has now also been shown to promote the synthesis of synaptic membranes, elevating the levels, per brain cell, of both the phosphatides and the specific pre- and post-synaptic proteins that characterize these membranes (19). DHA also increases the numbers of dendritic spines (20), and probably synapses, on hippocampal neurons, particularly on excitatory glutamatergic synapses. These effects, described below, can also be produced by eicosapentaenoic acid (EPA), another omega-3 fatty acid, but not by the omega-6 fatty acid arachidonic acid (AA) (21). They are considerably amplified if animals also receive two compounds that, with DHA, are present in mother's milk or infant formulas, i.e. uridine (19), a circulating pyrimidine which gives rise in brain to uridine triphosphate (UTP) and cytidine triphosphate (CTP) (22,23) and choline. It is thus possible that DHA affects cognition principally by promoting neurotransmission, and that it does so by increasing the numbers of certain synapses.

DHA and Uridine Increase Phosphatide and Synaptic Protein Levels in Gerbil and Rat Brain

Three circulating compounds are essential precursors in the synthesis of phosphatidylcholine (PC), the major phosphatide in neuronal membranes (1), as well as of phosphatidylethanolamine (PE), and, indirectly, by base exchange, of phosphatidylserine (PS): DHA; a uridine source; and a choline source. Each of these precursors is able to limit the overall rate of PC synthesis because its levels in brain are insufficient to saturate the brain enzymes that catalyze its utilization; moreover, the effects of giving all three together tend to be substantially greater than the summed effects of giving each alone. Uridine may also promote membrane synthesis via UTP, which activates P2Y receptors that promote neurite outgrowth (24), and DHA's effects may, as described above, also involve additional sites of action besides neuronal phosphatide synthesis. Perhaps surprisingly, when the three precursors are administered chronically, not only do brain levels of phosphatides – a lipid moiety – rise but also those of various pre- and post-synaptic proteins (19); moreover, structural changes occur – an increase in the number of dendritic spines, and thus synapses, on hippocampal neurons (20).

The utilization of DHA, uridine, and choline to form phosphatides like PC and phosphatidylethanolanine (PE) is mediated by the enzymes of CDP-choline cycle, or Kennedy

cycle (25). Phosphatidylserine (PS), the other main structural phosphatide, is formed by exchanging a serine molecule for the choline in PC or the ethanolamine in PE. Phosphatidylinositol (PI) synthesis also utilizes DAG and uridine, but different biosynthetic enzymes.

In the CDP-choline cycle, first choline is phosphorylated to phosphocholine by the enzyme choline kinase (CK); then CTP:phosphocholine cytidyl transferase (CT), transfers a cytidylylmonophosphate (CMP) moiety from CTP to the phosphorus of phosphocholine, yielding cytidylyldiphosphocholine (also known as CDP-choline, or citicoline). Much of the CTP that the human brain uses for this reaction derives from circulating uridine, hence brain CTP levels vary with plasma uridine concentrations (22). The third and last reaction, catalyzed by CDP-choline:1,2-Diacylglycerol choline phosphotransferase (CPT), bonds the phosphocholine of CDP-choline to the hydroxyl group on the 3- carbon of diacylglycerol (DAG), yielding PC. There are many types of DAG in the brain, differing in their two fatty acid constituents. DAG molecules that contain DHA are preferentially utilized for phosphatide synthesis (18). Once the new phosphatide molecule has been formed, this DHA can be removed by phospholipase A_2 and replaced by a different fatty acid, which need not be polyunsaturated (1). Hence, giving DHA can increase total membrane phosphatide levels without necessarily increasing steady-state membrane DHA contents. All three of the PC precursors must be obtained by brain entirely or in large part from the circulation; all three readily cross the blood brain barrier (23,26,27) and are metabolized by low-affinity brain enzymes to form PC.

Thus, choline administration increases brain phosphocholine levels in rats (28) and humans (29), because CK's Km for choline (2.6 mM) is much higher than usual brain choline levels (30-60 μM). Generally the second, CT-catalyzed reaction is most rate-limiting in PC synthesis, either because not all of the CT enzyme is fully activated by being attached to a cellular membrane or because local CTP concentrations are insufficient to saturate the CT (30). Thus, when brain CTP levels are increased by giving animals uridine (22), CTP's circulating precursor in human blood (31), PC synthesis is accelerated (19). The activity of CPT, the third enzyme, and the extent to which it is saturated with DAG, can also control the overall rate of PC synthesis, as has been demonstrated in, for example, permeabilized Hela cells exposed to glycerol-3-phosphate and acyl-CoA (32), or in PC12 cells extending neurites following exposure to the nerve growth factor (NGF) (24). In PC-12 cells, NGF increased DAG levels five-fold, CPT activity by 70%, and the incorporation of choline into PC by two-fold (33).

If rodents are given a standard diet supplemented with choline and uridine (as its monophosphate, UMP) and also, by gavage, DHA, brain PC synthesis rapidly increases (22), and absolute levels of PC per cell (DNA) or per mg protein increase substantially [(e.g., by 40-50% after several weeks of daily treatment (19)]. This treatment also increases the levels of each of the other principal membrane phosphatides, as well as of particular proteins (19, 20,21) known to be localized within presynaptic and postsynaptic membranes (for example synapsin-1, PSD-95 and syntaxin-3, but not a ubiquitously-distributed brain protein, β-tubulin). Moreover, it promotes the formation of dendritic spines by excitatory glutamatergic neurons in adult gerbil hippocampus (20), and improves hippocampus-dependent cognitive behaviors in gerbils and rats (c.f. ref. 34) (e.g., in aged animals or those reared in a socially-deprived environment). Providing supplemental DHA or UMP alone can also increase brain phosphatide levels and those of some of the synaptic proteins, but by considerably less than when all three precursors are presented.

Synaptic Phosphatides or Proteins are Not Increased by Omega-6 Fatty Acid Arachidonic Acid

In experiments designed to compare the effects on brain phosphatide levels of administering each of the three PUFAs in brain, the omega-3 fatty acids DHA and eicosapentaenoic acid

(EPA), and the omega-6 fatty acid arachidonic acid (AA), animals received by gavage one of the fatty acids daily for 4 weeks, and consumed a choline-containing diet that did or did not also contain UMP. Giving DHA without uridine increased PC, PI, PE and PS levels significantly, by 18%, 20%, 22%, and 28% respectively, throughout the brain (e.g. in cortex, striatum, hippocampus, brain stem and cerebellum). Giving EPA also increased brain PE, PS, and PI levels significantly, by 21%, 24% and 27%, respectively (21). In contrast, AA administration failed to affect brain levels of any of the phosphatides. Consuming the UMP-supplemented diet alone increased brain PS and PC levels significantly and enhanced the effects of DHA or EPA on all of the phosphatides. In contrast, when UMP was given with AA its effects were no greater than when it was given alone.

Giving the gerbils DHA (or EPA) alone also increased brain levels of all pre- or post-synaptic proteins examined, e.g. syntaxin-3, the postsynaptic density protein PSD-95; synapsin-1, actin, the metabotropic glutamate receptor 1, but failed to affect brain levels of the ubiquitous protein beta-tubulin. Giving UMP enhanced these increases in synaptic proteins. AA failed to affect levels of any of the proteins, nor to increase the effects of giving UMP alone

The mechanism that allows the omega-3 fatty acids DHA or EPA, given with UMP, to produce substantial increases in pre- and post-synaptic proteins may involve expressing the genes for these proteins: in a preliminary study, hippocampi of gerbils receiving DHA plus UMP for 4 weeks were found to contain elevated levels of the mRNA for the metabotropic-1 glutamate receptor (Cansev, Wurtman, unpublished observations), a protein located within dendritic spines; levels of this protein, assayed by Western blots, also increased.

Mechanisms that could underlie the differential effects of omega-3 and omega-6 PUFAs on synaptic membrane synthesis might include, among others, different efficacies for their uptakes into brain or their acylation; different half-lives in the circulation; different affinities for enzymes that control their incorporation into DAG and phosphatides; differences in the rates at which the PUFAs are removed from phosphatides by deacylation; the differential activation of genes encoding proteins that affect membrane synthesis (c.f. ref. 35); or the ability of AA but not DHA to be incorporated into phospholipids by the acetylation of 1-acyl-2-lyso-snglycerophospholipids and not solely via the Kennedy Cycle (36).

DHA Increases Hippocampal Dendritic Spines

Most dendritic spines, the small membranous protrusions extending from post-synaptic dendrites in neurons, eventually form synapses with presynaptic axon terminals. These structures compartmentalize post-synaptic responses, and their numbers are thought to reflect the density of excitatory synapses within regions of the central nervous system, e.g. the glutamatergic hippocampal synapses (37) which participate in learning and memory. Gerbils that received daily doses of DHA for 4 weeks (100 or 300 mg/kg, by gavage) exhibited increased dendritic spine density (i.e. the number of spines per length of dendrite) in CA1 pyramidal neurons; the increases were 12 percent ($p = .04$) with the 100 mg/kg/day dose, and 18 percent ($p < .001$) with the 300 mg/kg/day dose. These effects were amplified when gerbils received both DHA (300 mg/kg/day, by gavage, as above) and UMP (0.5%, via the standard choline-containing diet) for 4 weeks, DHA supplementation alone increasing spine density by 19 percent ($p < .004$) and administration of both precursors doing so by 36%. (Giving UMP alone did not affect dendritic spine density significantly; however, it did increase spine density when all dendritic protrusions were included for statistical analysis, including the filopodia, which are precursor forms of dendritic spines). The effect on dendritic spine density of giving DHA with UMP was already apparent after 1 week of treatment, and continued for as long as animals were treated (4 weeks). Giving DHA with uridine promoted cognitive behaviors in aged rats or animals reared in a socially-deprived environment (c.f. ref. 34,40).

Effects of DHA on Brain during Early Development

If pregnant rats receive – during the ten days prior to parturition and for the initial twenty days of lactation - daily doses of DHA by gavage and supplemental uridine (as UMP) via their diets, brains of their offspring exhibit neurochemical changes similar to those described above in adult animals: DHA alone produces small (20-30%) increases in each of the major phosphatides, while giving DHA plus UMP produces greater (50% or more) increases, per cell and per brain. Largest changes occur in phosphatidylinositol, the precursor of the second messengers inositol triphosphate and diacylglycerol, its levels more than doubling in animals whose mothers receive DHA plus UMP. Most of these increases result from supplementation during the postnatal period, since brains of offspring obtained and assayed at birth did not exhibit significant changes. Treating the mothers with DHA plus UMP also elevates brain levels of pre- and post-synaptic proteins (e.g., PSD95; synapsin-1; the metabotropic GluR-1 receptor; syntaxin-3) in the infant rat, and the numbers of hippocampal dendritic spines. Hence this treatment may affect the number of brain synapses formed during development. No data are available at present on how long these increases persist, nor on their possible functional or behavioral consequences. However it should be noted that, in other studies, brains of rats whose mothers received supplemental choline during embryonic days 11-17 exhibited, postnatally (days 15-34), increased expression of genes for brain proteins thought to be related to cognitive function (e.g., calcium-calmodulin-dependent protein kinase [CaMK]; insulin-like growth factor), and such animals have been shown to manifest lifelong improvements in memory performance (41).

Human breast milk contains both DHA and arachidonic acid, the levels of which depend on the mother's diet: Among women consuming cod liver oil, the DHA/AA ratio is about 4:1, while among those who instead consume corn oil, this ratio is only 1:1. Breast milk also contains choline and uridine, as such and as UMP, UDP-glucose, and UTP, but also as cytidine, CMP,CTP, - all converted to uridine in the human liver (31) - and as RNA, which is readily broken down to uridine in the newborn's intestine (42,43) Commercial infant formulas also include these compounds, however their uridine contents are not, in general, as great as those a breast-fed infant would obtain from the uridine- or cytidine-containing compounds (including RNA) in mothers' milk. Whether or not providing additional DHA uridine or choline would improve brain development in normal infants, or facilitate recovery from neonatal brain injury remains to be determined.

Acknowledgements

This work was supported in part by the National Institute of Mental Health (MH-28783) and the Center for Brain Sciences and Metabolism Charitable Trust.

References

1. Wurtman, RJ.; Cansev, M.; Ulus, I. Choline and its products acetylcholine and phosphatidylcholine. In: Lajtha, A., editor. Handbook of Neurochemistry. Springer-Verlag; Berlin: 2008. in press
2. Salem, N, Jr. Omega-3 fatty acid, molecular and biochemical aspects. In: Spiller, G.; Scala, J., editors. New Protective Roles of Selected Nutrients in Human Nutrition. New York: R. Liss; 1989. p. 109-228.
3. Eilander A, Hundscheid DC, Osendarp SJ, et al. Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: A review of human studies. Prostaglandins, Leukot and Essent Fatty Acids 2007;76:189–203.
4. Fleith M, Clandinin MT. Dietary PUFA and preterm and term infants: Review of clinical studies. Crit Rev in Food Sci and Nutri 2005;45:205–29.
5. Birch EE, Garfield S, Hoffman DR, et al. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Dev Med & Child Neurol 2000;42:174–81. [PubMed: 10755457]

6. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatydlcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: The Framingham Heart Study. Arch Neuol 2006;63:1545–50.
7. Kalmijn S, van Boxtel MP, Ocke M, et al. Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. Neurology 2004;62:275–80. [PubMed: 14745067]
8. Morris MG, Evans DA, Tangney CC, et al. Fish consumption and cognitive decline with age in a large community study. Arch Neurol 2005;62:1849–52. [PubMed: 16216930]
9. Huang TL, Zandi PP, Tucker KL, et al. Benefits of fatty fish on dementia risk are stronger for those without APOE ε 4. Neurology 2005;65:1409–14. [PubMed: 16275829]
10. Bongiovanni KD, DePeters EJ, van Eenennaam AL. Neonatal growth rate and development of mice raised on milk transgenically enriched with omega-3 fatty acids. Pediatr Res 2007;62:412–6. [PubMed: 17667849]
11. Yehuda S. Omega-6/omega-3 ratio and brain-related functions. World Rev Nutr Diet 2003;92:37–56. [PubMed: 14579682]
12. Kim HY. Novel metabolism of docosahexaenoic acid in neural cells. J Biol Chem 2007;26:18661–65. [PubMed: 17488715]
13. Lukiw WJ, Cui JG, Marcheselli VL, et al. A role for docosahexaenoic acid-derived neuroprotection in neuronal cell survival and Alzheimer disease. J Clin Invest 2005;115:2774–83. [PubMed: 16151530]
14. Montuschi P, Barnes PJ, Roberts LJ. Isoprostanes: markers and mediators of oxidative stress. FASEB J 2004:1791–800. [PubMed: 15576482]
15. Kawakita E, Hashimoto M, Shido O. Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. Neuroscience 2006;139:991–97. [PubMed: 16527422]
16. Darios F, Davletov B. Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. Nature 2006;440:813–17. [PubMed: 16598260]
17. Little SJ, Lynch MA, Manku M, et al. Docosahexaenoic acid-induced changes in phospholipids in cortex of young and aged rats: a lipidomic analysis. Prostaglandins, Leukot Essent Fatty Acids 2007;77:155–62. [PubMed: 17928211]
18. Richardson UI, Wurtman RJ. Polyunsaturated fatty acids stimulate phosphatidylcholine synthesis in PC12 cells. Biochem Biophys Acta 2007;1771(4):558–63. [PubMed: 17350887]
19. Wurtman RJ, Ulus IH, Cansev M, et al. Synaptic proteins and phospholipids are increased in gerbil brain by administering uridine plus docosahexaenoic acid orally. Brain Res 2006;1088:83–92. [PubMed: 16631143]
20. Sakamoto T, Cansev M, Wurtman RJ. Oral supplementation with docosahexaenoic acid and uridine 5′-monophosphate increases dendritic spine density in adult gerbil hippocampus. Brain Res 2007;1182:50–9. [PubMed: 17950710]
21. Cansev M, Wurtman RJ. Chronic administration of docosahexaenoic acid or eicosapentaenoic acid, but not arachidonic acid, alone or in combination with uridine, increases brain phosphatide and synaptic protein levels in gerbils. Neuroscience 2007;148:421–31. [PubMed: 17683870]
22. Cansev M, Watkins CJ, van der Beek EM, et al. Oral uridine-5′-monophosphate (UMP) increases brain CDP-choline levels in gerbils. Brain Res 2005;1058:101–8. [PubMed: 16126180]
23. Cansev M. Uridine and cytidine in the brain: their transport and utilization. Brain Res Rev 2006;52:389–97. [PubMed: 16769123]
24. Pooler AM, Guez DH, Benedictus R, et al. Uridine enhances neurite outgrowth in nerve growth factor-differentiated pheochromocytoma cells. Neuroscience 2005;134:207–14. [PubMed: 15939540]
25. Kennedy EM, Weiss SB. The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem 1956;222:193–214. [PubMed: 13366993]
26. Cornford EM, Oldendorf WH. Independent blood-brain barrier transport systems for nucleic acid precursors. Biochim Biophys Acta 1975;394:211–9. [PubMed: 1138930]
27. Marszalek JR, Lodish HF. Docosahexaenoic acid, fatty acid-interacting proteins and neuronal function: breastmilk and fish are good for you. Annu Rev Cell Dev Biol 2005;21:633–57. [PubMed: 16212510]

28. Millington WR, Wurtman RJ. Choline administration elevates brain phosphorylcholine concentrations. J Neurochem 1982;38:1748–52. [PubMed: 7077335]
29. Babb SM, Ke Y, Lange N, et al. Oral choline increases choline metabolites in human brain. Psychiatry Res 2004;130:1–9. [PubMed: 14972364]
30. Ross BM, Moszczynska A, Blusztajn JK, et al. Phospholipid biosynthetic enzymes in human brain. Lipids 1997;32:351–8. [PubMed: 9113621]
31. Wurtman RJ, Regan M, Ulus I, et al. Effect of oral CDP-choline on plasma choline and uridine levels in humans. Biochem Pharmacol 2000;60:989–92. [PubMed: 10974208]
32. Lim P, Cornell R, Vance DE. The supply of both CDP-choline and diacylglycerol can regulate the rate of phosphatidylcholine synthesis in HeLa cells. Biochem Cell Biol 1986;64:692–8. [PubMed: 3756002]
33. Araki W, Wurtman RJ. Control of membrane phosphatidylcholine biosynthesis by diacylglycerol levels in neuronal cells undergoing neurite outgrowth. Proc Natl Acad Sci USA 1997;94:11946–50. [PubMed: 9342342]
34. Holguin S, Huang Y, Liu J, Wurtman R. Chronic administration of DHA and UMP improves the impaired memory of environmentally impoverished rats. Behavioral Brain Research. 2008in press
35. Kothapalli KS, Anthony JC, Pan BS, et al. Differential cerebral cortex transcriptomes of baboon neonates consuming moderate and high docosahexaenoic acid formulas. PLoS ONE 2007;2:e370. [PubMed: 17426818]
36. Lands WEM, Inoue M, Sugiura Y, et al. Selective incorporation of polyunsaturated fatty acids into phosphatidylcholine by rat liver microsomes. J Biol Chem 1982;257:14968–72. [PubMed: 7174678]
37. Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. Nature 2004;429:761–6. [PubMed: 15190253]
38. Wang L, Albrecht MA, Wurtman RJ. Dietary supplementation with uridine-5′-monophosphate (UMP), a membrane phosphatide precursor, increases acetylcholine level and release in striatum of aged rat. Brain Res 2007;1133:42–8. [PubMed: 17184749]
39. Wang L, Pooler AM, Albrecht MA, et al. Dietary uridine-5′-monophosphate supplementation increases potassium-evoked dopamine release and promotes neurite outgrowth in aged rats. J Mol Neurosci 2005;27:137–46. [PubMed: 16055952]
40. Teather LA, Wurtman RJ. Chronic administration of UMP ameliorates the impairment of hippocampal-dependent memory in impoverished rats. J Nutr 2006;136:2834–7. [PubMed: 17056809]
41. Mellott TJ, Follettie MT, Diesl V, et al. Prenatal choline availability modulates hippocampal and cerebral cortical gene expression. FASEB 2007;21:1311–23.
42. Thorell L, Sjöberg, Hernell O. Nucleotides in human milk: sources and metabolism by the newborn infant. Pediatr Res 1996;40:845–52. [PubMed: 8947961]
43. Leach JL, Baxter JH, Molitor BE, et al. Total potentially available nucleosides of human milk by stage of lactation. Am J Clin Nutr 1995;61:1224–30. [PubMed: 7762521]