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Supplement 1. Translational and mechanistic studies of prenatal nutrients

Translational and other mechanistic studies of the nutrients have not been a prominent feature in their perinatal use, because these substances were already in general clinical use for other reasons. Vitamins A and D are in multivitamins because childhood blindness and rickets are produced by their severe deficiency. Omega-3 fatty acids were studied because of the possible cardiovascular benefit of diets that include fish. Folic acid was introduced after clinical observations of midline development defects related to its deficiency in pregnancy (14). Choline is the only substance to be studied as a direct result of translational research.

Studies on fetal brain development have emphasized folic acid's role in one-carbon metabolism, a pathway that also requires choline. This pathway is responsible for the methylation of DNA among other biomolecules and thus is a mechanism for epigenetic transmission of traits. Folic acid deficiency lowers several transcription factors, particularly Stat3, which is associated with decreased neurite development in cell culture (41).

Omega-3 fatty acids are accumulated in large amounts as constituents of cell membranes during fetal development. Most translational studies have shown positive effects on brain development. In cell culture, they enhance the differentiation of hippocampal neurons from embryonic stem cells and help maintain their survival. Docosahexaenoic acid (DHA) increases the expression of Growth Associated Protein 43 (GAP43), essential for neurite outgrowth. The mechanism responsible for the increase in symptoms of ADHD or later schizophrenia is unknown. Omega-3 fatty acids accelerate the inactivation and retard the recovery from inactivation of calcium and sodium channels in immature hippocampal neurons (45). This effect causes deceased neuronal excitability. Excitation early in development is critical for the development and strengthening of neuronal synaptic connections. Later in life the effect on excitability is hypothesized to be responsible for the anti-epileptic effect of the ketogenic diet, which is high in omega-3 fatty acids. A possible mechanism of the more beneficial reduction of preterm birth is the decreased production of prostaglandins E2 and F2alpha in decidual cells (46).

Most choline is synthesized into the cell membranes of the placenta and fetus. Choline also interacts with folic acid in one carbon metabolism (100). However, its translational study was motivated by its possible role in cholinergic neurotransmission. Cholinergic pathways from the midbrain do not reach the hippocampus and cerebral cortex until just before birth (101). However, choline at concentrations found in amniotic fluid is an alpha7-nicotinic receptor agonist on hippocampal inhibitory neurons (102). Initial studies in rodents showed that choline

supplementation during pregnancy enhanced the offsprings' spatial and temporal memory (103). Subsequent studies showed enhanced development of inhibition of hippocampal evoked responses to repeated auditory stimuli, a mouse analog of human P50 auditory sensory gating (44). Null mutation of *CHRNA7*, the gene that codes for the alpha7-nicotinic cholinergic receptor, abolished the effect of maternal choline supplementation on this inhibition, consonant with the *CHRNA7* genotypic effect of choline in human fetuses (43, 30, 75).

Agonism of alpha7-nicotinic receptors during fetal development facilitates transition from the embryonic membrane chloride transporter NKCC1 to the mature form of the chloride transporter KCC2, which increases the chloride gradient across the membrane and thereby lowers neuronal membrane potential. This transition switches GABA's activation of chloride channels from its fetal excitatory role to its inhibitory adult role (43). Alpha7-nicotinic receptor agonism similarly switches the fetal NMDA-glutamate receptor to mature AMPA-kainate-type receptors, which are faster and require lower levels of synaptic depolarization (104). Human post mortem studies show that neither the transition in chloride transporter nor the transition in glutamate receptors is complete in patients who have schizophrenia (105, 106).

The translational rodent model of maternal Vitamin D deficiency involves both dietary and light deprivation to lower Vitamin D levels without depleting calcium and phosphate levels. The resultant Vitamin D-deficient fetal brains are larger, with larger ventricles but thinner neocortices. Compared to normal, the deficient brains have more cell proliferation and lower levels of apoptosis. They are thus in a more embryonic state at birth. Cells that continue to proliferate in vitamin D-deficient cell cultures show less neurite outgrowth (107). At the more basic level, Vitamin D binds to a nuclear receptor that is part of the neurosteroid group. Among other factors, an interaction with Nurr1 is a possible mechanism of diminished development of dopaminergic neurons (47). In Vitamin D deficient animals, dopamine levels are normal but turnover appears to be altered. Vitamin D also facilitates the activity of low voltage (L-type) calcium channels, leading to increased neurofilament phosphorylation (48).

Translational models have identified effects of cytokines, particularly Interleulin-6 (IL-6), as the mediators between common maternal infections and the developmental risk for schizophrenia (108). These infections rarely enter the amniotic sac or invade the fetal brain. Instead, the maternal cytokine reaction causes invasion of the chorionic villi by Hofbauer cells, activated macrophages that attack the placenta as a foreign body, the same mechanism that underlies the immediate response to infection. Animal translational models inject double-stranded mRNA to mimic viral infection or lipopolysaccharides (LPS) to mimic bacterial. The effect on behavior is dependent upon which brain region is developing at the time of injection. Earlier infections affect the basal ganglia and produce hyperactivity; later infections affect the hippocampus and produce more cognitive deficits. At 12-16

week gestation human infection likely affects hippocampal and early neocortical development (109). Alpha7-nicotinic receptors in the peripheral nervous system, activated by the vagus nerve in adults, modulate the immune response (110). These receptors are also present on placenta cells (111) and TNF-activated macrophages (112). Maternal choline supplementation during the exposure to double-stranded mRNA diminishes some of the effects on the offsprings' behavior; the effect is abolished in *CHRNA* null mutants (48).

Other influences on the placenta include malnutrition, which induces cytokines including IL-6. (113). Maternal stress methylates several glucorticoid-related genes which results a hypo-responsive hypothalamic-pituitary axis and neurobehavioral changes in the fetus, but fetal alcohol exposure is associated with a hyper-responsive axis (7, 114). Recent GWAS studies also posit the role of the placenta in mediating maternal risk factors during gestation and subsequent schizophrenia in the offspring. GWAS for genes interacting between early life complications, including maternal infection, and schizophrenia identifies a set of genes orthogonal to the more common schizophrenia risk genes. These genes associated with both early life complications and later schizophrenia are largely expressed in the placenta and up-regulated by cellular stress, particularly in males (115).

Gestation in rodents, the most common laboratory model, differs considerably from the longer *in utero* development of humans. Nonetheless, biomarkers related to the mechanism of action of specific nutrients are being derived from animal studies to support clinical trials in human pregnancy to help establish the optimal dose and timing of maternal nutrient interventions. Such biomarkers might be particularly helpful for interventions like omega-3 fatty acids, where the desirable effects on preterm birth and wheezing might have different mechanisms and hence different biomarkers than the effects on the development of mental illnesses.

Supplement 2. Other interventions to decrease risk for mental illness

Infection, high body mass index (BMI), diabetes mellitus, anemia, depression, stress and anxiety, smoking and alcohol use, and other obstetrical complications are maternal risk factors associated with abnormalities in fetal brain development (2, 7-10, 115). Good maternal care including immunization, screening and treatment for anxiety and depression, blood sugar and weight monitoring, and encouragement of smoking and alcohol abstinence are all within the scope of prevention (116).

There are no studies of the effects of smoking cessation treatment during pregnancy on childhood development. Given the putative importance of nicotinic receptors for brain development, nicotine replacement therapy and varenicline might be avoided if the mother can respond to antidepressants or other anti-smoking remedies. Both nicotine and varenicline produce their anti-smoking effects by desensitizing nicotinic receptors, which might also prevent them from fulfilling their developmental role (117). Bupropion produced a 19% rate of abstinence in pregnancy, compared to 2% with placebo (118). The recent European Teratology survey found no evidence of congenital birth defects with varenicline, bupropion, or nicotine replacement therapy (119). A U.S. database reported a slightly increased risk of ventricular septal defects with bupropion. However, nearly half the women who received bupropion were smoking during pregnancy (120).

The effects of antidepressants, the chief agents used for both depression and anxiety, have been debated extensively. Risk for later autism spectrum disorder has been largely ascribed to confounding by the risk of maternal depression for later autism spectrum disorder (13). Although antidepressants slightly decrease gestation time compared to gestation times in the general population, babies born to women who are depressed and who have taken antidepressants have gestation times equal to those born to depressed women who do not take antidepressants (121-124). P50 sensory gating in infants of depressed and anxious women who took antidepressants was significantly more likely to show normal levels of inhibition, compared to those whose mothers did not take antidepressants (125). The finding suggests beneficial effects for the infant of the drugs, compared to no treatment, in maternal anxiety and depression.