Impact of Pregnancy Nutrition on Cognition

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Evidence shows that early nutrition can influence later mental performance, cognitive development, and behavior. Nutrition plays an important role in supporting structural and functional growth of the human brain from conception, through childhood and adolescence, and into adulthood.[1] Most of the energy is spent on supporting the propagation of action potentials, followed by postsynaptic signaling and maintenance of resting potentials.[2] A steady supply of macronutrients and micronutrients also is important for the synthesis of neurotransmitters, as well as their receptors and transporters, for the renewal and maintenance of the axonal cytoskeleton and its myelin sheath, for the growth of synaptic spines, and, as such, neural plasticity, and for neuronal survival.[1]

During the short period of 9 months, the initial "mother" cell gives rise to more than 100 billion nerve cells and a brain that weighs approximately 400 g when the child is born. During the first 4 years of life, the brain continues to grow, reaching the size of 1200 g at 3 years, which is only approximately 200 g less than that of an adult's brain. During the next 10–15 years, brain growth continues, involving different brain compartments in a slightly different way. For example, the thickness of the different regions of the cerebral cortex changes between the ages of 5 and 18 years at different paces, with the regions important for reasoning, planning, and social communication maturing last.

The idea that the diet of mothers could have an influence on long-term mental performance has major implications for public health practice and policy development and for our understanding of human biology, as well as for food product development, economic progress, and future wealth creation. Nutrients might serve as critical signals, acting directly or via coupling mechanisms on "receptors" in sensitive tissues.[3] Some programming events might have immediate effects on structural development of the brain (eg, on dendrite arborisation or glial cell growth with long-term consequences).

Although many epidemiological studies have uncovered associations between various macronutrients and micronutrients and cognitive performance and mental health,[4] the results of randomized clinical trials (RCTs)[5] less often support causal effects of nutrition on the brain and cognition. It is argued that most RCTs are too

short (lasting only a few months), too focused on a single micronutrient, or too small to detect modest effect sizes against the heterogeneous genetic and environmental background of the participating individuals.[5] Furthermore, the primary outcome measures, such as rating scales of cognition and mental health or even some of the cognitive tests, are perhaps too insensitive or may have low test-retest reliability. In this context, the use of various approaches to directly measure brain structure and function seems appealing.[1]

Methodologies for the Assessment of Brain Development

A number of techniques are available for the assessment of nutrition-related variations in brain structure and function (Table). With the exception of positron emission tomography (PET), it is possible to apply all of the methods mentioned from childhood onward.

Table. Methodologies to Explore Brain Development

Neuropsychological tests	Electrophysiological recording	Neuroimaging
Study of different domains to assess: • Intelligence and mental performance • Psychomotor development • Behavior maturation	**Visual and auditory acuity:** • Sweep VEP • Transient flash VEP • Pattern-reversal stimuli VEP • Steady state VEP • HOTV visual acuity • Sonksen-Silver acuity system • Teller acuity cards **Scotopic ERG** **EEG** **EEG/ERP**	**Brain structure and function:** • aMRI • fMRI • MEG • PET

aMRI=anatomical magnetic resonance imaging, EEG=electroencephalography, ERG=electroretinogram, ERP=event-related potentials, fMRI=functional magnetic resonance imaging, HOTV=single letters that are presented to the child using the Electronic Visual Acuity System,[6-8] MEG=magneto-encephalography, PET=positron emission tomography, VEP=visual evoked potential

Neuropsychological Development Assessment

Neuropsychological development is a heterogeneous process in which several critical periods are involved.[9] Therefore, the effects of nutrition on mental performance will depend on maturation stages and are easier to detect during and after each critical period. In addition, the neuropsychological tests should assess the specific neuropsychological domains (perceptual, motor, attention, learning and memory, and executive functions), instead of global cognitive performance.[9,10]

Recent papers reported some methodological concerns in studies about the effect of nutrition on mental performance, cognition, and behavior.[9,11] First, global measures of mental performance are possibly not sensitive enough, so specific effects of the nutritional intervention are hidden or masked. Second, for the assessment of nutritional effects, the specific approach is theoretically driven according to a specific biological mechanism. Moreover, it is necessary to pay special attention to cultural factors in order to compare similar neuropsychological tests administered in several countries. Another factor to consider is the practice/learning effect, especially if the intention is to test some improvement after a nutritional intervention.[12,13]

These suggest that any one test is not enough to detect significant changes in brain development because of a specific nutrient supplementation. Therefore, each study should have carefully designed, specific neuropsychological tools combining different neuropsychological domains to evaluate the potential effect of a nutrient, always taking into account the biological mechanism involved in the specific nutrient-effect that is explored.

Electrophysiological recordings can help us understand the cognitive functioning in children, especially because they can provide information about the “when” and even the “where” in the brain that these functions are taking place (Figure).[14]

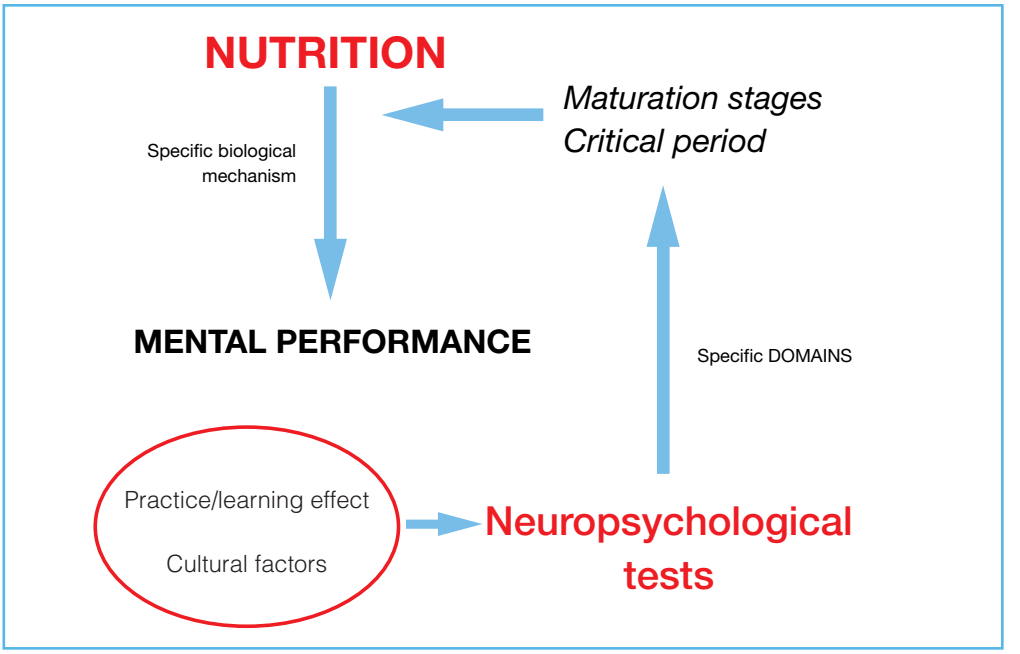

Figure. Electrophysiological recordings.

Visual Acuity

Visual development is incomplete at birth, particularly in premature infants. Maturation of the visual system, which includes neurological and ocular components, is influenced by many factors, such as prenatal and postnatal nutrition and postnatal visual stimulation.[15]

Evaluation by testing for visual acuity is performed in newborns (3–12 months), preverbal infants (12–24 months), and verbal infants.[16] Tests include preferential looking and visual tracking tests. Preferential looking is tested using static Teller acuity cards,[17-19] forced-choice preferential looking (FPL) or the Bébé vision test,[20,21] and other computerized infrared oculography, which allows testing in preverbal infants.[22] All methods employ a manual or automated system based on black and white patterns, usually vertical black and white bars or gratings on cards or a screen.

FPL, which is used in neonates up to 6 months of age, is based on the premise that neonates dislike boring visual stimuli and will tend to look at a pattern rather than a plain display. In FPL, infants are shown a display containing a pattern (the stimulus), and the observer decides where the stimulus is located, based on their observation of the infant's head and eye movements.[21] Because age causes the highest spatial

frequency resolved by a child to vary, the Operant Preferential Looking Test was adapted for use as a shorter screening procedure in children from as young as 6 months of age and up to 3 years, using the appropriate specific diagnostic-grating frequency for the age of the child.[23]

Visual evoked potential (VEP) testing[24] is used to assess communication between the eye and the brain and was adapted for use in preverbal infants as an automated test known as sweep VEP.[25] The sweep technique allows measurement of VEP in a few seconds, ensuring that the child's attention is maintained and stable fixation achieved for a sufficient time to complete the test, even in newborns.[25] However, as the test parameters are subjective and involve lengthy interpretation, sweep VEP mainly is used in the research setting.

An electroretinogram (ERG) is used to assess retinal function by measuring the difference in electrical potential between the anterior eye surface and the face in response to a visual stimulus.[26]

Electroencephalography/Event-Related Potentials (EEG/ERP)

EEG/ERP is a noninvasive method for assessing brain activity from scalp recordings. Researchers interested in cognitive development and brain functioning have used these techniques in their studies for decades. The applications of EEG/ERP are wide, including use to discriminate the effect of glucose or protein intervention on visual and auditory perception.[27,28] Other studies utilize EEG/ERP to analyze the effects of iron or iodine supplementation on working memory.[29] Some studies are exploring the effect of interventions with lemon balm, ginseng, or *Ginkgo biloba* on attention and vigilance, while other studies have explored EEG/ERP with other brain domains involved in problem solving, emotions, or decision making.

For instance, the development of attention networks were traced using the Attention Network Test (ANT)[30-32] and behaviorally[33] by EEG/ERP measures.[34] Both measures are proven to discriminate attention abilities as a function of age and training,[34-36] and even temperament.[37] Literature clearly indicates that both behavioral and EEG indices of ANT are a steady measure of the brain attention networks, especially in the case of the conflict resolution system. Within the NUTRIMENTHE EU Project, a protocol was developed to analyze the effect of early nutrition on attention and working memory at 8.5 and 9.5 years of age to detect long-term effects of dietary intervention with docosahexaenoic acid (DHA) and folate during pregnancy.

One of the most relevant parameters for analysis within the EEG/ERP examination is the N2 wave. This appears at the time that the individual is performing the action after processing the paradigm shown. The N2 is an early frontal negativity that is elicited during conflict and inhibition tasks[38] and is thought of as a marker for cognitive control processes, most notably conflict monitoring and detection.[39,40] The N2 is linked to affective, attentional, and cognitive factors that appear to play a role in the emergence of a range of mood and anxiety problems.[41,42] Thus, the N2 has the potential to serve as a neurophysiological marker for a biological diathesis of attention and temperamental factors associated with affective risk and resilience.[41,43] N2 also is useful for other cognition assessments (ie, using the working memory task developed in NUTRIMENTHE EU Project).

EEG/ERP also offered interesting neuroimaging, which helps locate exactly the place in the brain that is activated at the same time the children are trying to solve the paradigm proposed.

Neuroimaging Studies

Structural Magnetic Resonance Imaging (MRI)

MRI is a noninvasive technique that creates detailed three-dimensional pictures of the brain, each consisting of thousands of three-dimensional image elements called voxels. The most common structural MRI sequence produces T1-weighted images, which are characterized by a high contrast between gray matter and white matter. These images are analyzed using various computational algorithms that quantify, automatically and precisely, many different features, such as thickness of the cerebral cortex, volume of gray and white matter, and tissue densities.[44] In addition, other MRI sequences allow one to assess the microstructure of white matter (diffusion tensor imaging and magnetization transfer imaging) or the chemical composition of brain tissue (magnetic resonance spectroscopy).[1]

Functional Magnetic Resonance Imaging (fMRI)

For imaging brain function, the most commonly measured MRI parameter is the blood-oxygenation-level dependent (BOLD) signal. The BOLD signal reflects the proportion of oxygenated and deoxygenated blood in a particular brain region at a given moment. A strong correlation between the amount of synaptic activity and regional cerebral blood flow is why the BOLD signal is a good, albeit indirect, measure of brain function.[45] In the majority of fMRI studies, changes in BOLD signal are measured in response to various sensory, motor, or cognitive stimuli.

Therefore, only brain regions that respond to a particular set of stimuli are examined using the given paradigm.

Structural MRI and resting EEG procedures are used in healthy, unsedated infants as young as 7 days old,[46,47] while fMRI and event-related EEG and MEG (magneto-encephalography) are feasible on subjects aged approximately 5 years or older. In large studies (n=>100) and RCTs, structural MRI is the best imaging modality for evaluating the long-term effects of nutrition on the brain. In RCTs with modest sample sizes (n=<100), a combination of structural MRI and fMRI with EEG would provide the most comprehensive assessment of brain structure and function and, hence, offer insights into possible brain mechanisms underlying the effect of nutrients on cognition and mental well-being. Overall, brain imaging offers a rich armamentarium of acquisition and analysis tools for the quantitative in vivo assessment of the effects of nutrition on the human brain.[1]

Update of Findings

A lack of clarity and little consensus still remain regarding the role of several nutrients, such as proteins, long-chain polyunsaturated fatty acids (LCPUFAs), B vitamins, minerals and other nutrients in neurodevelopment, mental performance, and mental illness. Many animal studies have demonstrated that changes in dietary nutrients can alter brain morphology, as well as its biochemical functions, but human studies have not offered clear evidence of the specific effect of each nutrient during early life, except in some specific cases.

Protein

Protein deprivation can cause direct deleterious effects on the brain, such as loss of brain weight, altered hippocampal formation, impairment of neurotransmitter systems, and changes in protein phosphorylation.[48] Undernourished children (under 3 years of age) usually have lower development, behavior, and school achievement, and supplementation studies have shown benefits on their development.[49] These benefits are attributed to energy and protein supplementation rather than to micronutrients.[50] No data are available from large prospective follow-up studies on the possible effect of different levels of protein intake early in life on later neurodevelopment for well-nourished infants. Breastfed infants have lower protein intake and show lower insulin-like growth factor-1 (IGF-1) levels.[51] Children who were breastfed in the early weeks of life had a significantly higher IQ in childhood compared to formula-fed children exposed to high protein intakes during the first 12 months of life who had higher plasma concentrations of total and free IGF-1.[52,53]

IGF-1 is involved in brain development, ranging from neuroprotection after neuronal damage to neurogenesis, myelination, synaptogenesis, and dendritic branching.[54-57] Furthermore, overexpression of the IGF-1 gene determines an increased brain size,[58] whereas targeted IGF-1 gene deletion stunts brain size.[59] These studies prove that IGF-1 is associated with brain development in childhood, but detailed investigation of influencing factors still is needed.

Long-chain Polyunsaturated Fatty Acids (LCPUFAs)

Animal models have shown that maternal diets deficient in n-3 and n-6 LCPUFAs alter the accretion of these fatty acids in the neonatal cortex, which results in changes in neurotransmitter metabolism and learning impairments.[60] DHA deficiency leads to reduced dendritic arborisation[61] and impaired gene expression for regulation of neurogenesis, neurotransmission, and connectivity.[62,63]

A growth spurt in the gray matter of the human brain occurs during the 3rd trimester of pregnancy and the first postnatal months, with a large increase in the cerebral content of arachidonic acid (AA; 20:4 n-6) and DHA.[63] Adverse outcomes associated with insufficient intake of long-chain omega-3 fatty acids during pregnancy include intrauterine growth retardation, delayed or suboptimal depth perception,[64] adverse neurodevelopmental measures,[65] residual deficits in fine motor skills, speed of information processing in infants,[66] and irreversible deficits in serotonin and dopamine release. Higher maternal intake of DHA results in higher maternal plasma levels and thereby increased DHA transfer to the fetus.[67] Thus it seems an appropriate prenatal and postnatal supply of LCPUFAs is essential for normal fetal and neonatal growth, neurological development, and functional maturation, including learning and behavior. However, the long-term effects of LCPUFA supplementation on the neurocognitive outcome and mental performance of these infants remain unclear.

A large observational study described beneficial effects on cognitive development in children whose mothers consumed seafood during pregnancy.[65,68] Supplementation studies during pregnancy and/or lactation have not demonstrated clear evidence of beneficial effects of LCPUFAs on visual acuity (ERG and VEP), stereoacuity, or neurodevelopment.[69-77] Only three trials have reported long-term effects of supplementation to date.

Helland et al report a better performance in the Kaufman Test (K-ABC) in the supplemented group compared to control at 4 years of age, but this effect was not observed in IQ at 7 years. The authors also report a significant positive correlation between IQ at 4 years and DHA levels in infant plasma at the 4th week of life, as

well as an association between maternal DHA levels in 35 weeks of gestation and IQ in children at 7 years of age.[71,72] The NUHEAL trial reported no differences in neurological outcome of children at 4 years and 5½ years between children born to mothers receiving fish oil supplements and those who did not, but they did demonstrate better neurological optimality scores in children at 5½ years with increasing DHA levels in cord blood.[78] Moreover, children whose mothers had higher DHA percentages in erythrocyte phosphatidylethanolamine at delivery were more likely to have a Mental Processing Composite score of the K-ABC over the 50th percentile at 6.5 years.[79] Despite these contradictory results, a sufficient availability of DHA during the perinatal brain growth spurt is widely considered mandatory for normal cognitive, visual, and motor development.[80]

Recently, it was demonstrated that polymorphisms of FADS1 and FADS2 (fatty acid desaturase gene variants) are closely related to important changes in fatty acid metabolism linked to brain development.[81] Koletzko et al[82] showed a consistent and significant association of rare single-nucleotide polymorphism alleles with lower amounts of DHA in red blood cell phospholipids of pregnant women. A modulation of DHA status during pregnancy by frequently occurring FADS genotypes is possibly of major relevance for child outcomes.

As previously mentioned, several cohort and randomized control studies showed positive but also null associations between LCPUFA intake and status in the prenatal and postnatal period and developmental outcomes in early childhood. However, more studies are required to explore the effects of FADS gene variants in populations with different ethnic backgrounds, lifestyles, and dietary habits, and to investigate in greater depth the interaction of FADS gene variants, diet, and clinical end points of such developmental outcomes.

Folate

Normal brain development and function also depend on the active transport of folate across the blood-brain barrier. Supplementation of pregnant mothers with folate significantly decreases the incidence of developmental defects, including neural tube defects (NTDs), conotruncal heart defects, and cleft lip/palate. Relative folate deficiency is not uncommon and does not appear as harmful in most pregnancies. The risk appears the greatest if the mother's homocysteine levels, which are genetically influenced, are elevated. Hyperhomocysteinemia (HtHcys) is related to fetal toxicity because of DNA alterations, hypomethylation, or insufficient synthesis of DNA because of structural damage in the genes implied in the DNA synthesis.

The most common mutation of the enzyme methylenetetrahydrofolate reductase (MTHFR) gene is 677C→T. The 677TT homocygosis determines a low MTHFR activity, which is related to NTDs and HtHcys. Vitamin B_{12} and folate deficiencies are common during childhood[83] and may have an acute effect on the central nervous system (CNS) via hypomethylation, inhibiting the synthesis of methionine and forming S-adenosylmethionine. This in turn inhibits methylation reactions throughout the CNS involving proteins, membrane phospholipids, and DNA and metabolism of neurotransmitters, such as dopamine, norepinephrine, serotonin, and melatonin.[84] Another potential mechanism of how folate could relate to mental performance is facilitating DHA accretion by the fetus.[85]

Probably the most significant effect at present related to folate status during pregnancy and behavior is the emerging results from Generation R within the NUTRIMENTHE EU Project. Children of mothers who did not use folic acid supplements during the 1st trimester of pregnancy had a higher risk of total problem behavior. Use of a folic acid supplement protected from both internalizing and externalizing problems, even when adjusted for maternal characteristics such as age, national origin, educational level, and psychopathology.[86] In addition, data from the Generation R Study recently have shown that low levels of maternal folate are associated with smaller head circumference and smaller transcerebellar diameter in the fetus.

In conclusion, well-designed supplementation studies with long-term follow-up, examining all new confounding factors and combining the new different methodologies, are required to advance the knowledge of optimal nutrition during early life, promote optimal neurodevelopment, and prevent deficiencies and other pathologies.

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Q & A

Q: One of the reasons that the Institute of Medicine's Committee on Weight Gain and Pregnancy was worried about having obese pregnant women not gain enough weight or to even lose weight was an old finding that ketonemia might impair cognitive development in the child. Have you heard of that hypothesis?

Dr Campoy: Yes.

Q: Can you talk a little bit about that because it is really murky. To my knowledge, the research basically stopped in the 1970s or 1980s, and we found really insufficient information either way. I just wonder how you might approach studying that given that ketonemia is pretty hard to measure in the mother.

Dr Campoy: Yes, it is a very interesting topic. It is shown that obese adolescents have problems in the development of executive functions, which are the last domain in the steps of brain development. This alteration is linked to the metabolic and inflammatory changes in obese patients [Verdejo-García A et al. *Obesity* (Silver Spring). 2010;18:1572-1578]. The most critical windows for brain development are

established during prenatal life. It is shown that babies born from diabetic mothers could have an impaired neurodevelopment. After the diagnosis of gestational diabetes, the women are treated in the 3rd trimester of pregnancy, including pharmacological treatment as needed, and principally dietetic treatment and physical activity recommendations. This intervention is shown to have beneficial effects for the mother and fetus development.

What happens with a child born from an obese mother? More than 30% of obese pregnant women develop gestational diabetes (diabesity), becoming the worst metabolic situation for brain development. But in the case of obese pregnant women, actually no treatment is implemented and no dietary advice is regularly recommended. Sometimes the obese mothers are only advised about recommended weight gain during pregnancy. So, the metabolic and inflammatory status is maintained and probably these insults will determine an abnormal fetal programming effect, which is similar to that already seen during diabetic pregnancies, phenotypically manifested by newborns large for gestational age. However, we suspect that brain development also is affected in the offspring of obese mothers. The problems eventually are no longer as studied.

Obese mothers normally have a very bad diet, and so most of them have lipid and micronutrient deficiencies, such as folic acid or iron, among others. Significantly lower levels of serum iron and transferrin saturation (the ratio of serum iron to total iron-binding capacity) were found in obese as compared to nonobese adult volunteers, and fat mass was shown as a significant negative predictor of serum iron concentration [Menzie CM et al. *J Am Diet Assoc.* 2008;108:145-148].

Anemia during pregnancy is linked to brain development impairment, but the long-term effects were not studied extensively. Limited evidence suggests that iron supplementation during pregnancy may positively influence children's psychomotor development during the first 2 years of life, whereas it does not seem to alter their mental development or behavior [Szajewska H et al. *Am J Clin Nutr.* 2010;91: 1684-1690].

It is necessary to analyze iron supplements during pregnancy individually, because supplemental iron during pregnancy will increase the oxidative stress and could cause even worse problems for fetal neurodevelopment. This is a topic for new studies.

We actually are involved in assessing this topic in the cohort of the PREOBE Study. We are exploring the effects of obesity and diabetes, and mothers' nutritional status during pregnancy on their offsprings' neurodevelopment. The assessment of these

effects on neurodevelopment is very difficult, because many other confounder factors are involved. It also is necessary to examine long-term outcomes. At present, data from other studies already published are inconsistent.

Q: The comprehensive review of the broad spectrum of methodology that you presented perhaps illustrates the difficulty that we have. We do not really know what to measure and how. While it is very plausible that metabolic insults during the very rapid development of the brain early on should have an impact on long-term structured function, we really have very limited understanding of that. We obviously have some examples of dramatic insults, malnutrition of B_{12} deficiency in vegans, or high saturated fat or protein dietary intake.

If you think about the variation of normal, such as introduced by variation of nutritional habits, we really have very little knowledge and understanding as to how that would impact brain development and function.

What is the opportunity moving forward? Should we learn from the whole story of the omega-3 fatty acids, where the field was advanced by animal experimentation, where we had specific hypotheses, where we had chemical hypotheses, omega-3 fatty acids, resins, and photo receptors, and then we had primate studies showing effects and specific studies in humans based on that, which I think draw forward?

Or should we embark on broader fishing expeditions where, for example, we collect metabolic profiles in pregnant women or at-birth infants and then we take that to the broad spectrum of outcome markers in children or adolescents, and then based on that, we formulate hypotheses, or what is your vision? How do we progress in this sort of murky area?

Dr Campoy: Well, it is a really interesting question, because it is a major topic and a major problem. It is really difficult to assess the effect of nutrition during pregnancy and early life on long-term neurodevelopment, based only on the results of different neuropsychological tests available in each country. This could achieve important data, but with the results possibly influenced by many other confounder factors. Actually, new technologies will give us the opportunity to advance in the knowledge of how early nutrition impacts on brain development. However, there is still very little data of reference. For example, only a few studies are available with fMRI or with MRI in children.

If we use nutritional and metabolic biomarkers combined with these new techniques for neurological assessment, we could have a chance. MRI could allow us to analyze structural changes in the brain, depending on early nutrition and fMRI

functional changes, such as after nutritional interventions. The combination of these techniques (neuropsychological tests battery, VEP, EEG/ERP, MRI, fMRI, etc) will help us in future studies to understand the structural and long-term functional effects of early nutrition on brain development.

Future studies to demonstrate the long-term effects of early nutrition on brain development, using these methodologies, should include not only normal and healthy pregnancies, but also babies born to mothers with very common metabolic diseases, such as diabetes or obesity. Studies in children or adolescents also are needed, and the new procedures will prove extremely useful in understanding the brain effects after nutritional interventions.