Unraveling the Molecular Mechanisms of the Neurodevelopmental Consequences of Fetal Protein Deficiency: Insights From Rodent Models and Public Health Implications

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ABSTRACT

Fetal brain development requires increased maternal protein intake to ensure that offspring reach their optimal cognitive potential in infancy and adulthood. While protein deficiency remains a prevalent issue in developing countries, it is also reemerging in Western societies due to the growing adoption of plant-based diets, some of which are monotonous and may fail to provide sufficient amino acids crucial for the brain's critical developmental phase. Confounding variables in human nutritional research have impeded our understanding of the precise impact of protein deficiency on fetal neurodevelopment, as well as its implications for childhood neurocognitive performance. Moreover, it remains unclear whether such deficiency could predispose to mental health problems in adulthood, mirroring observations in individuals exposed to prenatal famine. In this review, we sought to evaluate mechanistic data derived from rodent models, placing special emphasis on the involvement of neuroendocrine axes, the influence of sex and timing, epigenetic modifications, and cellular metabolism. Despite notable progress, critical knowledge gaps remain, including understanding the long-term reversibility of effects due to fetal protein restriction and the interplay between genetic predisposition and environmental factors. Enhancing our understanding of the precise mechanisms that connect prenatal nutrition to brain development in future research endeavors can be significantly advanced by integrating multiomics approaches and utilizing additional alternative models such as nonhuman primates. Furthermore, it is crucial to investigate potential interventions aimed at alleviating adverse outcomes. Ultimately, this research has profound implications for guiding public health strategies aimed at raising awareness about the crucial role of optimal maternal nutrition in supporting fetal neurodevelopment.

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The developmental origins of health and disease theory embodies the idea that suboptimal environmental conditions in early life predispose to chronic noncommunicable diseases in adulthood, such as cancer, cardiometabolic disease, and neuropsychiatric disorders (1). An illustrative case is the cohort of individuals born to mothers who were pregnant during the Dutch famine of 1944 to 1945. At the age of 50 to 60 years, they performed worse on cognitive tests and were more likely to develop schizophrenia (2–4), an outcome that bore striking resemblance to a wider demographic of adults living in Anhui province, China, who experienced famine after the spring of 1959 (5). Nutritional deficiency that arises from either a caloric deficit or inadequate dietary nutrients remains a prevalent environmental stressor for the fetus and a major cause of intrauterine growth restriction (IUGR). IUGR, characterized by fetal weight below the 10th percentile for gestational age (6), represents a clinical condition with established links to delayed intellectual development (7) and increased susceptibility to mental health problems in adulthood (8).

The predominant cause of IUGR in Western societies stems from fetal amino acid deficiency, primarily associated with placental dysfunction (9). Studies with primates and monochorionic twins have demonstrated that reduced amino acid transport across the placental barrier precedes the onset of IUGR (10–12). Another cause is maternal protein deficiency, a prevalent concern in low-income countries (13). However, despite improvements in staple foods since the 1970s (14), protein deficiency in pregnant women in developed countries is more pervasive than anticipated. For example, 1 in 8 pregnant women in the United States fails to meet the newly established protein requirements for the second and third trimesters of pregnancy (Figure 1) (15–17).

The resurgence of dietary protein deficiencies is multifactorial. Firstly, there is the continual rise in the adoption of plant-based diets, which are health-beneficial but often lacking in protein quantity, digestibility, and quality (18). Among the 20 amino acids, 9—histidine, lysine, methionine, phenylalanine, threonine, tryptophan, and the branched-chain amino acids (BCAAs) isoleucine, leucine, and valine—are exclusively derived from the diet. Pregnant women who adhere to monotonous plant-based and vegan diets are particularly susceptible to inadequate intake of at least some essential

amino acids crucial for fetal development (19–22). Another cause of recurrent amino acid deficiency is obesity, which is associated with chronic calorie-rich, unvarying diets (23). The rising incidence of bariatric surgery, especially among young women with obesity, heightens the concern about potential protein deficiency during pregnancy (24,25). Finally, the current economic landscape makes protein-rich foods, such as meat, seafood, and certain fruits and vegetables, difficult to afford for people on low incomes. A population-based Spanish study showed that pregnant women of lower socioeconomic status were more likely to have hypoproteinemia (26).

Protein accretion is crucial for optimal brain development. Amino acids function not only as the fundamental building blocks for proteins but also as precursors for neurotransmitters, hormones, and enzymes. The first 1000 days of life comprise a meticulously orchestrated sequence of events encompassing neurogenesis, migration, differentiation, synaptogenesis, apoptosis, and gliogenesis, which shape the brain's structural foundation and set the boundaries for cognitive capacities in adulthood (Figure 1) (27). During this critical period, the brain exhibits remarkable plasticity, which places the fetus at risk of misprogramming if it is exposed to external stress such as nutritional deprivation (28–30). Confounding factors in human nutritional studies have impeded a comprehensive understanding of how protein deficiency during pregnancy affects neurodevelopment. Indirect evidence from preterm infants only suggests a correlation between fetal protein availability and childhood neurobehavioral performance (31,32), implying that a fetal deficit in protein may diminish cognitive abilities. Furthermore, it is unclear whether fetal protein deficiency may also predispose to neuropsychiatric disease in adulthood, as has been observed on multiple occasions in individuals who experienced acute prenatal caloric restriction (Table 1) (4,5,33).

To address how fetal protein deficiency may lead to adverse neurological outcomes, we sought to review mechanistic data derived from rodent models, with an emphasis on the involvement of neuroendocrine axes, the influence of sex and timing, and epigenetic modifications and cellular metabolism, before proposing perspectives on how the field can move forward.

NEUROANATOMICAL AND BEHAVIORAL CONSEQUENCES

Efforts to evaluate the impact of a nutritional factor, or its absence, in human cohorts predominantly rely on outcomes derived from psychosocial performance and intelligence tests, which are susceptible to a multitude of nonnutritional variables, such as socioeconomic status, education, and parenting. Furthermore, the exact dietary patterns in cohorts of pregnant women and their offspring vary considerably, which impedes researchers' ability to isolate the effects of dietary factors unequivocally. In contrast, dietary exposure in animal models can be thoroughly controlled and conducted under standardized conditions, which makes them particularly useful for nutritional research (34). Moreover, effects can be studied during embryonic, fetal, and neonatal stages. Research on brain development and behavioral end points following protein restriction has relied heavily on mouse and rat strains (Figure 1).
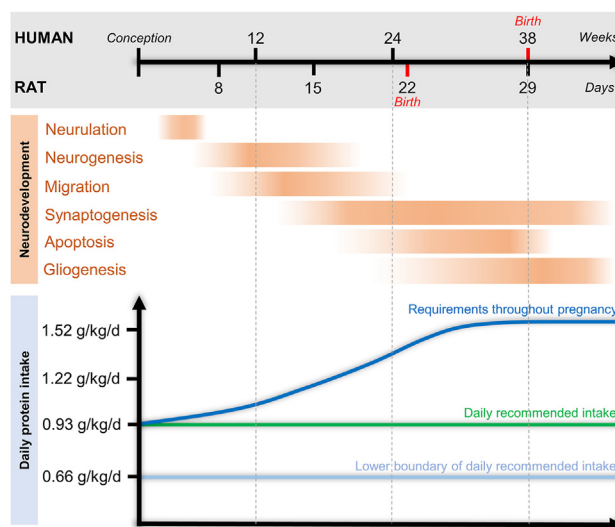

Figure 1. Illustration of key developmental events in rat and human brains, highlighting protein intake requirements for human fetal development. Rodents, chosen for their ease of use, shorter life span, and well-documented dietary needs, serve as valuable models. However, it is crucial to note their comparatively premature birth compared with humans. A newborn rat mirrors a human fetus at the end of the second trimester, while a human newborn aligns with a rat at postnatal day 7 (123). The period of neurogenesis in the rat spans from G10 to G20, peaking between G12 and G16, akin to the end of the first trimester to the start of the third trimester in humans. Recent advancements in protein requirement assessments during pregnancy utilizing the indicator amino acid oxidation method reveal higher needs: 1.22 to 1.52 g/kg/day at 16 and 36 weeks of pregnancy, respectively, compared with 0.93 g/kg/day in nonpregnant women (15,16). G, gestational day.

Most studies have employed a model of moderate protein restriction by feeding pregnant dams an isocaloric diet with 8% to 10% protein content, while control groups received a diet containing around 20% protein. More stringent models further reduced the protein content to 4% to 7% (Table 2). Others extended restriction until weaning or incorporated a period prior to conception. Low-protein diets reduce maternal amino acid levels and their transfer across the placenta to the fetus and to suckling pups during lactation (35–37). Despite hyperplasia and upregulation of amino acid transporters, the placenta is unable to restore adequate amino acid transfer to the fetus when the deficiency persists throughout gestation (38–41). Consequently, the offspring have lower birth weights and an altered physiology that attempts to compensate for the sustained amino acid deficiency (42–44).

The repercussions on neuropsychological performance of adult animals that were transiently exposed to a low-protein diet are diverse. A first consistent observation is the increased prevalence of anxiety-like behavior, as evidenced by various assessments such as ultrasonic vocalization after maternal separation, open-field, the elevated plus maze, and forced swim tests (45–52). Anxiety is a complex behavior that is primarily governed by the limbic system—the thalamus, hypothalamus, basal ganglia, hippocampus, and amygdala—and the hypothalamus-pituitary-adrenal (HPA) axis via glucocorticoid signaling (53). Elevated corticosterone levels in adult

Table 1. Exemplary Studies of the Impact of Gestational Malnutrition or Famine in Nonhuman Primates and Humans, Respectively, on Neurodevelopment, Cognitive Performance, and Associated Susceptibility to Neuropsychiatric Disorders in Adulthood

Analyzed Subjects	Neurodevelopmental and Behavioral Phenotype	Source
Epidemiological/Field Studies		
595 71- to 74-Year-Old Men and Women Exposed vs. Nonexposed to Prenatal Famine[a]	Self-perceived cognitive problems ↑ in women exposed during late gestation and ↑ in men exposed during early gestation Consultancies with health care practitioner for cognitive issues ↑	(125)
297 56- to 59-Year-Old Men and Women Exposed to Prenatal Famine vs. 737 Healthy Participants[a]	Selective attention abilities ↓	(3)
9 Patients With Schizophrenia vs. 9 Healthy Participants Exposed to Prenatal Famine, and 9 vs. 9 Not Exposed to Prenatal Famine[a]	Intracranial volume ↓ in patients with schizophrenia exposed to prenatal famine than those without exposure	(126)
6417 ≥45-Year-Old Adults Exposed to Prenatal Famine vs. Nonexposed Individuals[b]	Cognitive status, word recall, visuospatial episodic memory ↓	(127)
People Diagnosed With Schizophrenia (n = 4597) From 1971 to 2001[b] vs. Total Population and Birth Records	Adjusted risk of schizophrenia of those born during famine ↑	(5)
83 Mothers With CZS-Affected Children	% of mothers with CZS-affected children ↑ when low-protein intake (protein intake ref = women between 19 and 59 years)	(112)
130 Women in 4 Rural Guatemala Villages	Reading, knowledge numeracy at 22–29 years ↑ and educational achievement ↑ when completed primary school for those who received extra protein (6.4 g/100 mL) during prenatal period and first 2 years	(128)
Baboon Studies		
Baboons at 90% Term Following Exposure to 30% MNR From 16% to 90% of Gestation	At 90% of gestation: HPA axis overactivation due to ↑ plasma cortisol and ACTH NPY ↑, GR ↑, pGR ↑, POMC ↓, pSTAT3 ↓ Brain serotonergic expression ↓ and neurons ↓ IGF and mTOR system ↓ Perturbated neurodevelopmental processes and metabolism	(129–133)
Juvenile Baboons Born to MNR Mothers (30% Caloric Restriction, Gestation + Lactation)	At 3.3 years of age (before onset of sexual maturity): Motivation ↓ Associative learning in males ↓, in females ↑ Accuracy working memory performance ↓, impulsivity ↑	(134)

ACTH, adrenocorticotropic hormone; CZS, congenital Zika-virus syndrome; GR, glucocorticoid receptor; HPA, hypothalamus-pituitary-adrenal; IGF, insulin-like growth factor; MNR, maternal nutrient restriction; mTOR, mechanistic target of rapamycin; NPY, neuropeptide Y; pGR: phosphorylated glucocorticoid receptor; POMC, pro-opiomelanocortin; pSTAT3: phosphorylated STAT3; STAT3, signal transducer and activator of transcription 3.

[a]Dutch famine (1944–1945).
[b]Chinese famine (1959–1961).

rats gestationally exposed to protein deficiency have been reported repeatedly (45–47) and suggest alterations in HPA axis development. Juvenile rats had downregulated glucocorticoid and mineralocorticoid receptors in the amygdala (54). Individuals exposed to the Dutch famine prenatally and those born prematurely also had increased odds of experiencing anxious and depressive behavior, although no discernable impact on cortisol levels was observed in these cohorts (55–57).

Increased anxiety may also originate from the improper development of limbic regions and subsequent miscommunication within or between them. In rats, gestational protein deficiency impaired neuronal differentiation and dendritogenesis in the bed nucleus of the stria terminalis (45), a small structure downstream of the amygdala known for its involvement in anxiety and addiction in humans (58). In the amygdala, Nätt et al. found that decreased Npy1r (neuropeptide Y receptor 1) messenger RNA due to the suppressive effect of

early growth response factors (e.g., EGR1) may be responsible for increased anxiety in male rats (52). Another plausible explanation for this behavioral anomaly is impaired serotonergic signaling, which is strongly implicated in anxiety. Maternal protein deficiency reduced substrate affinity for the serotonin 5-HT$_{1A}$ receptor in the hippocampus of adult female offspring (59). This observation echoes findings from experiments conducted in the 1990s that showed an altered hippocampal neuronal circuitry due to impaired serotonergic signaling (60,61). Additionally, gestational and lactational protein deficiency induced overexpression of dopamine-related genes and increased numbers of tyrosine hydroxylase and dopaminergic neurons in the ventral tegmental area and prefrontal cortex, respectively, in adult mice. This increased the dopaminergic-driven locomotor response to cocaine and caused hyperactive behavior (62). Collectively, the data suggest that developmental protein deficiency can adversely impact the HPA axis, limbic system, and higher brain

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Table 2. Cellular and Behavioral End Points in Preclinical Rodent Studies Involving Gestational and/or Lactational Protein Restriction

Animal	PR Model	Time Window	Evaluation	Cellular Outcomes	Behavioral Outcomes	Sex	Source
SD Rats	23% vs. 9%	Gestation	P35	Impaired inhibitory network in anterior cingulate cortex, with: Microglia activation ↑ Oxidative stress ↑ Neuroinflammation ↑	NA	♂	(113)
CF1 Mice	20% vs. 8%	Gestation + lactation	8–18 weeks F1 + F2 generation	PET glucose PFC ↓ PFC transcriptome ↓ 21 dysregulated autism genes	Social motivation ↓ Recognition memory ↓	♀ and ♂	(70)
SD Rats	20% vs. 8%	Gestation	G17	Hypothalamic genes dysregulated Altered mitochondrial activity Methylation ↓ Neuronal progenitors ↑	NA	♀ and ♂	(83)
SD Rats	21% vs. 16%–18% vs. 10% vs. 5%	Gestation + lactation	6 weeks in F0, F1, and F2 generation	Dopamine ↑ Antioxidants ↓ Altered serotonin system	Reflexes ↓ Learning and memory ↓	NA	(135)
SD Rats	20% vs. 8%	Gestation + lactation Gestation or lactation	P12, P16	Hypothalamic energy pathways disrupted Dysregulation neurodevelopmental genes	NA	♂	(43)
Wistar Rats	17% vs. 6%	Gestation	P7, P14, 16 weeks	BNST neuronal dendritogenesis ↓ (nor)-epinephrine and DOPA in amygdala ↓ 11β-HSD2, GR, MR and type 1 CRH-R ↓ Corticosterone levels ↑	Anxiety-like behavior ↑	♂	(54,136)
Wistar Rats	20% vs. 10%	Gestation	P21, P97-112	5-HT$_{1A}$ receptor function in female hippocampus ↓ Serotonergic cell bodies in the rostral raphe and 5-HT metabolism unaltered	Anxiety-like behavior ↑ Stress sensitivity ↑ Stress-induced anorexia	♀ and ♂	(59)
MF1 Mice	18% vs. 9%	Pre-implantation period (G0-G3.5) or gestation	G12.5, G14.5, G17.5, 4–20 weeks	BCAAs in uterine fluid ↓ Blastocyst megalin expression ↑ Blastocyst mTORC1 signaling ↓ NSC proliferation ↓ Neuronal differentiation and cortical thickness ↑	Anxiety-like behavior ↑	♀ and ♂	(87,91,93)
Sv129 Mice	20% vs. 8%	Gestation + lactation	10 weeks	Egr1/2/4 genes upregulated in amygdala Downregulated Npy1r in amygdala of males only	Anxiety-like behavior ↑ in ♂ only	♀ and ♂	(52)
BL6 Mice	20% vs. 8%	4 weeks prior to mating + gestation + lactation	8–12 weeks	Clock genes dysregulated	Anxiety-like behavior ↑ Energy expenditure ↓ Circadian clock perturbed	♂	(49)
SD Rats	20% vs. 8%	3 weeks prior to mating + gestation	P0	Bdnf expression ↓ PKA/cAMP pathway activation ↓	NA	♀ and ♂	(99)
Wistar Rats	20% vs. 8%	Gestation + lactation	P90	Hippocampal Bdnf and Zip268 expression ↓ Neurogenesis ↓	Learning and memory ↓ Exploration ↓	♂	(69)
African Striped Mice	19% vs. 10%	Gestation + lactation (+ after weaning or not)	8 weeks	NA	Aggressivity ↑ Anxiety-like behavior ↑ Impaired learning	♀ and ♂	(51)
SD Rats	20% vs. 8% (± MD)	3–4 weeks prior to mating + gestation + lactation	G19, P0, P21	Proliferation ↑, methylation ↓ Dcx ↓ Gfap ↑ in neural stem/progenitor cells Hippocampal expression Insr ↓ Nestin and Igf2 ↑ at P21	NA	♀ and ♂	(98)
CF1 Mice	20% vs. 9%	5 days prior to mating + gestation + lactation	5–9 weeks	NA	Impaired social, risk, motivational, and exploratory behavior	♀ and ♂	(50)

Fetal Protein Deficiency and Neurocognitive Impairment

Table 2. Continued

Animal	PR Model	Time Window	Evaluation	Cellular Outcomes	Behavioral Outcomes	Sex	Source
Wistar Rats	17% vs. 6%	Gestation	16 weeks	Hippocampal CA3 dendritic atrophy Cytoarchitecture DG and CA1 unaltered	Learning and memory	♂	(137)
Wistar Rats	20% vs. 10%	Gestation + lactation	P17–22 and P90-96	Hippocampal progenitors ↓	Impaired memory Depression-like behavior	♂	(48)
Wistar Rats	20% vs. 10%	Gestation ± lactation	P90–220	Corticosterone levels ↑	Impaired learning Motivation ↓ Anxiety-like behavior ↑ in females and ↓ in males	♀ and ♂	(46,47)
Wistar Rats	20% vs. 8%	Gestation + lactation	P82–86	Cortical glutamatergic pathways, hormonal secretion and synaptic remodeling hypothalamus altered	NA	♂	(138)
Swiss Albino Mice	17% vs. 7%	6 weeks prior to mating + gestation + lactation	P7, P14, P60	Granule cells and EGL thickness ↓ Cerebellar PCs ↓	Delayed motor development	♀ and ♂	(139)
SD Rats	20% vs. 8%	Gestation + lactation	P35, P180	Hypothalamic insulin signaling and nutrient sensing ↓ Receptors and TFs in lipid metabolism ↓ 5-HT-regulated food intake ↓	Hyperphagia	♂	(140,141)
BL6 Mice	18% vs. 8.5%	Gestation + lactation	18–20 weeks	Dopamine circuitry VTA and PFC ↑ Hypomethylation *Cdkn1c* *Cdkn1c* expression ↑	Hyperactivity Altered reward processing Locomotor response to cocaine ↑	♂	(62)
Wistar Rats	20% vs. 10%	Gestation	G19	Brain lipids and DHA ↑	NA	NA	(45)
SD Rats	20% vs. 8%	Gestation (± lactation)	P0, P5, P12, P16	Impaired cell differentiation Delayed leptin surge AgRP and αMSH fibers in PVN ↓	NA	♂	(78)
Wistar Rats	20% vs. 6%	G8/G15–P28	P28	Protein levels in hippocampus and cerebral cortex ↓ BDNF and IGF-1 levels in hippocampus ↓	Impaired learning and memory	♀ and ♂	(67)
SD Rats	23.4% vs. 6%	Prenatal, postnatal, or chronic	P30, P90	Delayed GAD67 interneuron development	NA	♂	(142)
CD1 Mice	18% vs. 9%	G0–G12	G12	Brain AgRP, NPY, POMC, and LeptinR isoforms ↓	NA	NA	(82)
SD Rats	25% vs. 6%	5 weeks prior to mating + gestation	P90	Volume and neurons in hippocampal CA1 ↓ Volume pyramidal layer ↓	NA	♂	(68)
Wistar Rats	17% vs. 8%	Gestation + lactation	P20	Hypoglycemia and hypoinsulinemia VMN volume ↑ Neurons VMN and PVN ↑ NPY neurons in ARC ↓	NA	♂	(77)
Wistar Rats	20% vs. 8%	Gestation ± lactation	P90	Brain vascularization ↓	NA	♀ and ♂	(143)
Wistar Rats	20% vs. 5%	G0–G14	G15–P63	Transient ↓ neurogliogenesis, cortical thickness, synaptogenesis, and apoptosis	NA	♀ and ♂	(86)

The percentages indicate the relative protein amounts in the control and protein-restricted group diets. The studies are arranged chronologically based on their publication year, starting with the most recent.

5-HT, 5-hydroxytryptamine (serotonin); 11β-HSD2, 11-beta-hydroxysteroid dehydrogenase-like 2; AgRP, agouti-related peptide; ARC, arcuate nucleus; BCAA, branched-chain amino acid; BDNF, brain-derived neurotrophic factor; BNST, bed nucleus of the stria terminalis; CA, cornu ammonis; cAMP, cyclic adenosine monophosphate; Cdkn1c, cyclin dependent kinase inhibitor 1c; CRH-R, corticotropin-releasing hormone receptor; DG, dentate gyrus; DHA, docosahexaenoic acid; DOPA, dopamine; EGL, external germinal layer; G, gestational day; GAD67, glutamic acid decarboxylase 67; GR, glucocorticoid receptor; IGF-1, insulin-like growth factor 1; MBP, myelin basic protein; MD, methyl donor; MR, mineralocorticoid receptor; αMSH, α-melanocyte-stimulating hormone; mTORC1, mechanistic target of rapamycin complex 1; NA, not available; NPY, neuropeptide Y; NSC, neural stem cell; P, postnatal day; PC, Purkinje cell; PET, positron emission tomography; PFC, prefrontal cortex; PKA, protein kinase A; POMC, pro-opiomelanocortin; PR, protein restriction; pSTAT3, phosphorylated STAT3; PVN, paraventricular nucleus; SD, Sprague-Dawley; TF, transcription factor; VMN, ventromedial nucleus; VTA, ventral tegmental area.

structures to such an extent that it lowers the threshold for anxious behavior later in life. However, the relative contribution of each component remains unclear.

Learning and memory impairments have also been observed consistently across studies, suggesting dysfunction of the hippocampus and higher cortical regions. The latest neuroimaging techniques in humans revealed that fetal undernutrition reduces gray and white matter volume (63), which are correlated with life-long neurobehavior alterations (64). Optimal gray matter configuration hinges on a precisely timed transition between cycling neural stem cells, expanding the progenitor pool, and their subsequent exit from the cell cycle to contribute to neuronal differentiation (65). Consequently, premature cell cycle exit can result in cellular hypoplasia and precocious neurogenesis (66). Adult animal offspring that experienced developmental protein deficiency had fewer hippocampal progenitors, fewer neurons in the cornu ammonis 1 (CA1), and a reduced pyramidal layer volume (48,67,68). Reduced levels of BDNF (brain-derived neurotrophic factor) and IGF-1 (insulin-like growth factor 1), molecules that stimulate neuronal differentiation and survival, could underlie these observations (67). Pérez-García et al. showed that adult rats that experienced perinatal protein restriction and displayed these molecular and cellular alterations in the hippocampus underperformed on memorization tasks (69). Interestingly, a recent study showed that perinatal protein restriction in mice had transgenerational effects, impairing social motivation and recognition memory in F1 and F2 generations. The authors found reduced glucose metabolism in the prefrontal cortex and identified 21 autism-associated genes in an overall downregulated transcriptome (70).

Finally, fetal malnutrition may also contribute to the growing incidence of obesity and type 2 diabetes (71). Beyond a sedentary lifestyle and an imbalanced diet, increased susceptibility to metabolic diseases could also result from fetal misprogramming of the brain network that governs appetite, reward, and energy homeostasis (72). The hypothalamus is the central integrator of hunger and satiety signals to control appetite, relying on interconnected nuclei that sense leptin and gut peptides. Two neuronal populations in the arcuate nucleus play pivotal roles: the orexigenic AgRP (agouti-related peptide)/NPY (neuropeptide Y) neurons, inhibited by leptin, and the anorexigenic pro-opiomelanocortin (POMC) neurons, activated by leptin to produce α-melanocyte-stimulating hormone (αMSH). Binding to MC3/4 receptors in the paraventricular nucleus suppresses food intake and increases energy expenditure (73).

A crucial step in hypothalamic development unfolds early in life. In rodents, approximately half of the arcuate nucleus–POMC neurons at gestational day 13 (G13) transdifferentiate to generate the NPY neurons in the subsequent days (74). Gestational protein restriction disrupts this process of neurogenesis and cell fate determination (75,76), leading to fewer NPY neurons and attenuated projections from the arcuate nucleus to the paraventricular nucleus (77–79). This is manifested in hyperinsulinemia, increased body weight, hyperleptinemia, and altered leptin responsiveness, which are hallmarks of metabolic syndrome, in adult offspring (78,80,81). Another study found that altered expression of genes encoding NPY, POMC, and leptin receptor isoforms was evident in the

protein-deprived fetal brain as early as G12 (82). Employing RNA sequencing, our research group found that gestational protein restriction led to dysregulation in 6.5% of the total number of detected genes in the hypothalamus of G17 rats (83). Network analysis assigned these genes to cellular metabolism, RNA processing, oxidative phosphorylation, and apoptosis (Figure 2).

THE ROLE OF SEX AND TIMING

Female animals have often been excluded from preclinical studies due to the challenges posed by hormonal fluctuations associated with the menstrual cycle, which introduce increased variability and confounding results. However, their inclusion offers the opportunity to explore the etiology of sex-specific differences in neuroanatomy and behavior in human cohorts comprised of individuals who experienced the same conditions of dietary restriction (64). For example, men exposed to starvation in early pregnancy had smaller brain volumes and a higher likelihood of developing affective psychosis, whereas women did not (84,85). Some rodent studies have described sex-specific effects of fetal protein deficiency on neuroanatomy and behavior (Table 2). Hippocampal 5-HT$_{1A}$ receptor dysfunction was exclusive to females (59), and malnourished females exhibited higher brain synaptic density (86). Females' performance on stress tests was poorer than that of males (87), and females showed increased behavioral despair (46,50). In contrast, Nätt et al. discovered that male mice subjected to perinatal malnutrition showed more anxiety-like behavior than females (52). Transcriptome analysis of the prefrontal cortices of mice exposed to perinatal protein deficiency revealed numerous dysregulated genes that were not shared between the sexes despite similar behavioral phenotypes (70). Other studies have shown comparable responses in both sexes.

Thus, it remains inconclusive whether fetal protein deficiency affects brain development differently in males and females and the extent to which it may contribute to exacerbated neurocognitive impairments in either sex. The effects may be confined to specific brain regions or behaviors. Potential influencing factors include sex hormones, discordant neurodevelopmental timing, epigenetics, and divergent coping mechanisms (52,88). Additionally, discrepancies that arise from the use of different strains and species cannot be disregarded. A multiomics analysis revealed substantial differences in the molecular and behavioral responses to the same protein-restricted diet among 3 adult mouse strains (89).

In terms of timing, a noteworthy finding was that individuals who endured either the Dutch or the Chinese famine during the first trimester of pregnancy had a higher likelihood of suffering from schizophrenia (3–5). This suggests that the earliest stages of neurogenesis respond negatively to protein deprivation or that transient protein deficiencies, however brief, continue to impact amino acid supply even after protein uptake returns to normal. This raises the question of the time required for recovery from preconceptional amino acid deficiency, particularly if expectant mothers only transition to a protein-rich diet after pregnancy has been confirmed—often several weeks after conception—and therefore how long a fetus could be exposed to the delayed effects of deficiency. The importance
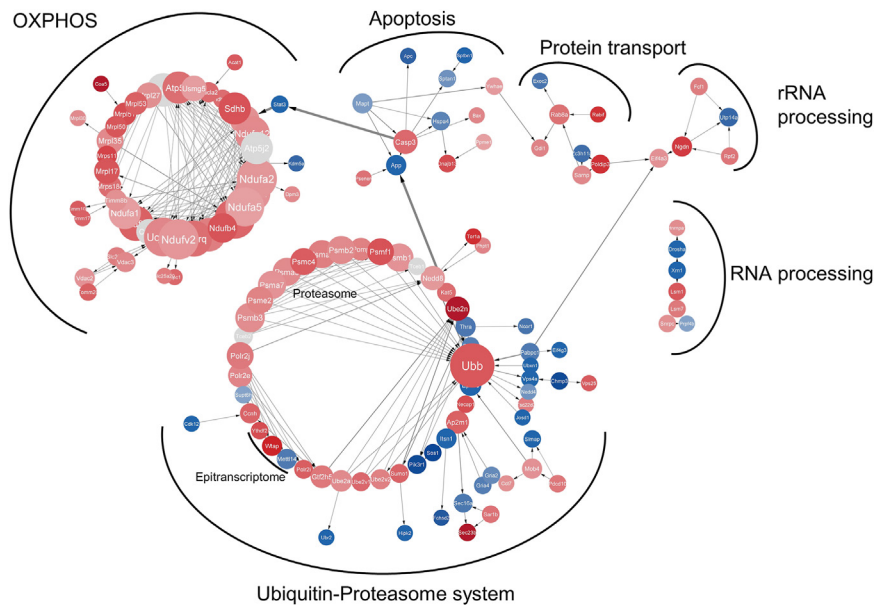
Figure 2. Network analysis of bulk RNA sequencing data from hypothalami dissected from G17 fetal rats exposed to an 8% protein-restricted diet during gestation. Utilizing Cyto-Scape version 3.9.1 against the *Rattus norvegicus* reference genome (stringency of 0.80), the full STRING network was plotted. Employing STRING functional enrichment, 361 dysregulated genes were categorized into 6 major clusters (red: upregulated, blue: downregulated). Within the cell metabolism cluster, proteasome components were upregulated and linked to the ubiquitin hub, overseeing protein transfer to the proteasome. Concurrently, most genes governing mitochondrial OXPHOS exhibited upregulation, consistent with previous Western blot findings. The upregulation of STAT3, known for releasing the suppression of proapoptotic genes like caspase-3 in cancer cells (124), suggests analogous molecular mechanisms during brain development [dataset from (83)]. G, gestational day; OXPHOS, oxidative phosphorylation; rRNA, ribosomal RNA; STAT3, signal transducer and activator of transcription 3.

of preconceptional nutrition in healthy infant development is exemplified by the necessity of supplementing with folic acid to prevent neural tube defects (90); commencing 1 month after conception is already past the period of most beneficial effects. At least one human cohort study reported that individuals exposed to the Dutch famine preconceptionally had smaller total brain volumes than their unexposed counterparts (85). It would certainly be interesting to investigate the effects of preconceptional protein deficiency in animal models.

That also prompts the question of how prolonged a protein deficiency must be to exert detrimental effects on brain development. Even a brief exposure to a low-protein diet limited to the first 3.5 days of gestation—the preimplantation period—sufficed to induce hyperactive and anxiety-like behavior as well as short-term memory deficits in adult mouse offspring (87,91). This remarkably short period of protein restriction reduced BCAA levels in blastocysts and blocked glucose metabolism, the primary energy source at this stage. In embryonic stem cells cultured from G3.5 blastocysts, MAPK (mitogen-activated protein kinase), ERK1/2 (extracellular signal-regulated kinase), and mTORC1 (mechanistic target of rapamycin complex 1) signaling pathways were dysregulated (92,93). Cell-tracing and immunohistochemical studies up to G12 showed reduced progenitor proliferation coupled with increased neuronal differentiation in the basal ganglia and cortex. This shift occurred without compensatory apoptosis, resulting in thicker cortices (91).

These findings emphasize the importance of maintaining adequate amino acid levels from the very onset of neurodevelopment and even preceding conception, challenging the conventional notion that adequate protein intake is only crucial later in pregnancy.

MOLECULAR MECHANISMS

What are the underlying molecular mechanisms by which amino acid deficiency disrupts neurodevelopment? A longstanding assumption posits that endocrine signals, which mirror maternal physiology and external conditions, traverse the placenta to reach the fetus, where they modify epigenetic marks, activating or silencing genes and thereby influencing cellular programs and the structural blueprint of the brain (29). Enzymes responsible for establishing and erasing epigenetic marks rely on substrates and cofactors such as NAD, FAD, acetyl-CoA, vitamins, and SAM (S-adenosylmethionine), which are derived from nutrients and their metabolites (94,95). The best-known is the one-carbon metabolic pathway and involves methyl donors like folate, betaine, choline, and methionine, providing methyl groups to SAM—the universal methyl donor for DNA and histones (96) (Figure 3). Alternative methyl donors include amino acids such as serine, glycine, and histidine (97). Data on how fetal amino acid deficiency reshapes the cellular epigenome and influences neurodevelopmental pathways remain fragmented, but epigenetic end points are increasingly being incorporated into research studies.

We reported that supplementing the diet of pregnant rats with methyl donors increased hippocampal neural stem cell proliferation in offspring but not when combined with protein restriction, suggesting that both amino acids and micronutrient-derived methyl donors are crucial for hippocampal neurogenesis (98). Vucetic *et al.* demonstrated that the reduction in dopaminergic neurons following perinatal protein restriction in mice resulted from hypomethylation and subsequent overexpression of the cyclin-dependent kinase inhibitor 1c (62). This prevented neural progenitors from exiting the cell cycle, thereby providing a mechanistic clue to the etiology of an altered dopaminergic circuitry in neuropsychiatric disorders such as schizophrenia. In another study, the absence of anxiety-like behavior in female mice, compared with males fed a low-protein diet during development, was suggested to be due to increased methylation of the *Npy1r* gene in the female amygdala. This maintained *Npy1r* expression despite elevated *Egr1* levels, which suppressed *Npy1r* in males (52). Reduced
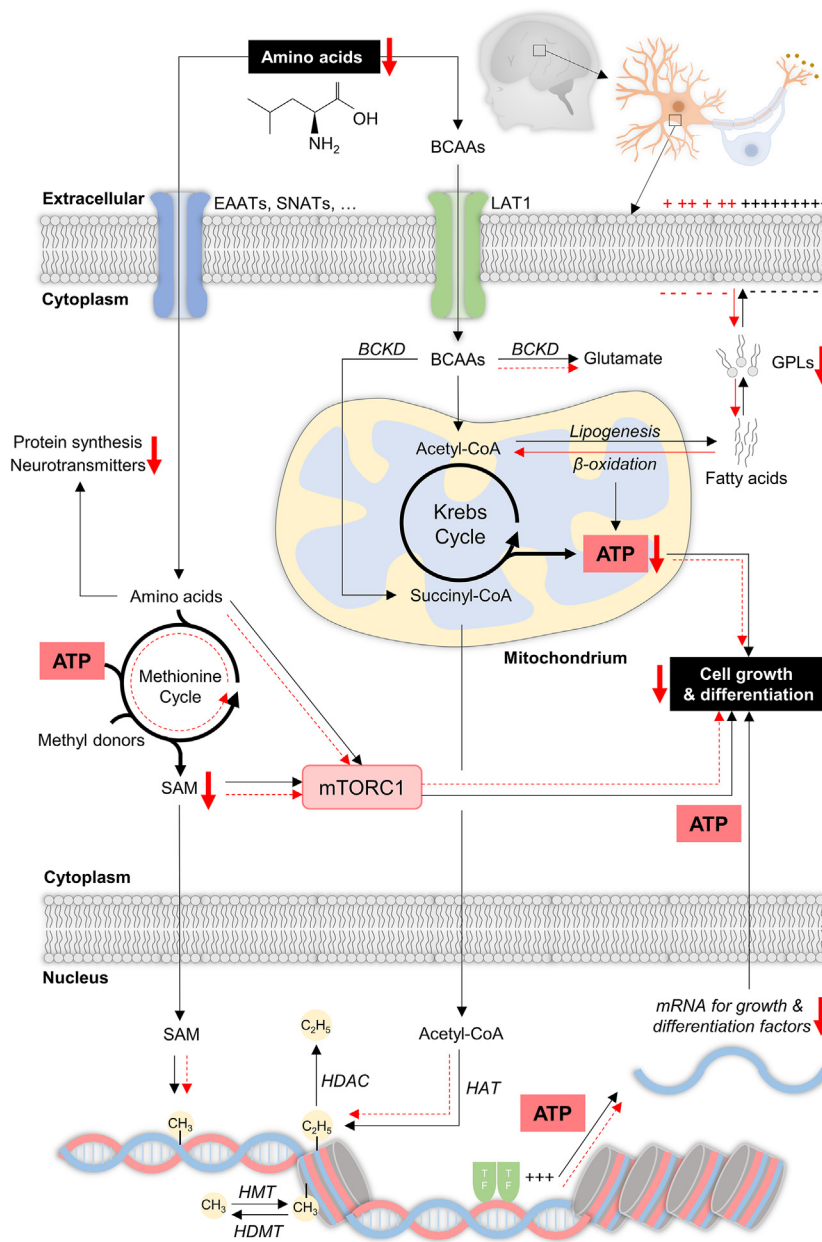
Figure 3. Concise overview of amino acid roles in neural precursor cell metabolism. Black lines denote physiological pathways, while red arrows indicate dysfunction upon cellular amino acid deprivation. LAT1 plays a vital role in BCAA uptake by neural precursors during development. This influences the growth, differentiation capacity, survival, and proper function of neural progenitors in the developing brain. ATP, adenosine triphosphate; BCAA, branched-chain amino acid; BCKD, branched-chain α-ketoacid dehydrogenase; EAAT, excitatory amino acid transporter; GPL, glycerophospholipids; HAT, histone acetyl transferase; HDAC, histone deacetylase; HDMT, histone dimethyl transferase; HMT, histone methyl transferase; LAT1, L-type amino acid transporter 1; mTORC1, mechanistic target of rapamycin complex 1; SAM, S-adenosyl methionine; SNAT, sodium-coupled neutral amino acid transporter; TF, transcription factor.

brain *Bdnf* expression in neonatal rats exposed to a low-protein diet prenatally was found to be a consequence of repressive histone methylation (99). In the hypothalamus, we identified a hypomethylated *Pomc* promoter following perinatal protein restriction, which potentially contributes to altered feeding behavior in young rats (78). Interestingly, this dietary regimen also reduced levels of the epitranscriptomic mark *N6*-methyladenosine in the fetal hypothalamus, thus potentially affecting messenger RNA stability and turnover (83) (Figure 2). The involvement of the epitranscriptome in the mechanistic origins of metabolic disease is an emerging area of investigation (100).

Given the striking parallels with the effects of maternal psychosocial stress on offspring cognitive and neuropsychological outcomes (59,101), potential targets could also be retrieved from gene databases derived from these studies. For example, increased methylation of the glucocorticoid receptor gene *NR3C1* in adult offspring exposed to maternal psychological stress appears to be a key mechanism through which perinatal stress leaves a lasting mark (29,102). This mechanism may explain HPA axis dysregulation in protein deficiency.

In tandem with epigenetic mechanisms that modify gene expression, amino acids interact with cellular metabolism (103). The increased influx of amino acids is sensed by

mTORC1 (104), the principal modulator of cell growth and differentiation (105). This activation induces a conformational change and the recruitment of cofactors, initiating RNA translation and activating essential signaling pathways. To meet the increased energy demands, a neural progenitor cell undergoes mitochondrial biogenesis and a metabolic shift from glycolysis to oxidative phosphorylation to upscale ATP (adenosine triphosphate) production (106,107). BCAAs and their metabolites thereby serve as substrates for acetyl-CoA and succinyl-CoA to fuel the Krebs cycle (Figure 3).

Recently, Knaus *et al.* (108) obtained important clues as to how cellular BCAA deficiency affects neuronal development through these molecular pathways and causes neurological disease. They had previously identified patients who exhibited microcephaly and autism spectrum disorder who harbor mutations in the *SLC7A5* gene. This gene encodes the LAT1 (L-type amino acid transporter 1) (109), prominently expressed in the blood-brain barrier and in neural cells (110,111). Its pivotal role in blood-brain barrier–dependent amino acid uptake was confirmed in mice lacking SLC7A5 in endothelial cells (109). However, selectively deleting *Slc7a5* in cerebrocortical neurons of perinatal mice revealed that cellular amino acid uptake is equally essential for neuronal maturation (108). The resulting deficiency of BCAAs failed to drive ATP generation, which is crucial for promoting neuroblast differentiation (106). To compensate, lipid catabolism increased, and fatty acids derived from triacylglycerols served as an alternative fuel source for the Krebs cycle. This shift reduced their availability for membrane glycerophospholipid production, which led to cortical neuroarchitectural defects (108). This phenotype is consistent with the observed 2-fold reduction in brain lipids, including ω-3 polyunsaturated fatty acids, in G19 rat fetuses exposed to a low-protein diet (45). Consequently, utilizing lipids for energy production to counteract amino acid deficiency means that they are no longer available for other processes dependent on them, such as cell membrane formation and myelinogenesis. This dual impact may exacerbate the adverse effects of protein-poor diets on brain development.

CONCLUSIONS AND PERSPECTIVES

Protein deficiency, whether resulting from compromised placental amino acid transport or malnutrition, represents a prevalent concern in pregnancy and neonatal care because it potentially impedes optimal neurocognitive development during infancy. Insights obtained from rodent studies suggest that misconfigured neuroendocrine axes and persistent alterations in neuroanatomy contribute to dysfunction of brain regions that have been implicated in cognitive abilities. Specific outcomes are contingent upon the severity and duration of the insult and could vary as a function of the asynchronous development of different brain regions and their distinct amino acid and energy requirements, as well as be influenced by genetic predisposition and external factors like infection (112). Fetal protein deficiency may also impact mental health in the long-term, mirroring the increased susceptibility to neuropsychiatric disorders in adults who experienced famine during prenatal life. However, while animal data hint at such a connection, direct, concrete evidence in humans is missing.

Rodent studies, while informative, warrant caution in extrapolating complex behavioral traits to humans. In addition, studies of protein deficiency tend to center on a single anatomical brain region, typically the hippocampus, to explain specific behavioral changes. Such one-to-one relationships seem improbable. Rather, anxiety, impaired learning, memory deficits, and altered reward-seeking behavior likely represent distinct phenotypic outcomes that arise from disrupted neural networks spanning multiple brain regions and their intercommunication. At the cellular level, abnormalities may manifest quantitatively (e.g., cell types, cell numbers, synapses, dendrites) or functionally (e.g., impaired neurotransmission, receptor dysfunction, interneuron types, excitation-inhibition imbalance). Presumably, protein deficiency impacts most, if not all, of these processes. At the molecular level, epigenetics and cellular metabolism emerge as primary pathways through which protein deficiency imprints lasting effects on brain development. While a wealth of data exist for the neuronal component, our understanding of its impact on other cell types such as oligodendrocytes, astrocytes, and microglia remains limited. To our knowledge, only one study has revealed that gestational protein restriction in rats induces microglia to a state of hyperactivity, elevating oxidative stress and neuroinflammatory markers in adult stages (113). In addition, exploring the role of the placenta as a fetal caretaker in abnormal nutritional conditions deserves more attention (114,115).

The challenge ahead is to untangle the interrelationships among these anomalies to gain a holistic view of how fetal protein deficiency ultimately affects human intellectual capacities and behavior. This pursuit includes deciphering the extent to which these adverse effects may or may not be reversible and identifying the optimal timing for interventions. While inconclusive, the potential of citrulline and arginine to stimulate fetal growth in the case of IUGR is promising (116–118) and may serve as an example of a simple but effective therapy. However, the assessment of its beneficial effects on neurodevelopment and behavior later in life requires further exploration and evaluation.

One of the solutions to enhance our understanding of how amino acids affect the molecular machinery of the cell, epigenome, (epi)transcriptome, and developmental pathways can be achieved by the use of multiomics technologies at the single-cell level across various brain regions. Employing cell-targeted genetics will play an important role in distinguishing direct and indirect effects. The integration of neurobehavioral end points into mammalian models that better match the timing of neurodevelopmental events in human gestation, such as nonhuman primates, will contribute to bridging the gap between rodents and humans. In experimental studies conducted with baboons, maternal nutrient restriction yielded phenotypes remarkably similar to those observed in rodents subjected to a protein-restricted diet (Table 1). Innovative models like brain organoids offer the advantage of being able to test a diverse range of nutritional stressors on neurogenesis without interference from other environmental factors [e.g., (119)]. Additionally, exploration of rare genetic disorders related to amino acid metabolism may shed further light on the role of specific enzymes and the dependency of certain amino acids in neurodevelopmental processes (109,120,121). Moreover, it is imperative to thoroughly investigate how the timing

of nutritional deficiencies and sex influence phenotypic outcomes.

In summary, this review underscores the critical role of adequate protein intake before and during pregnancy, as well as lactation, in supporting optimal fetal brain development and mitigating the risk of neurocognitive impairments in offspring. It emphasizes the importance of incorporating food frequency questionnaires into public health studies, a practice that has been largely overlooked, and underscores the importance of shaping uniformity in national dietary guidelines with regard to the consumption of plant-based diets during pregnancy (122).

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REFERENCES

1. Barker DJP (2007): The origins of the developmental origins theory. J Intern Med 261:412–417.
2. Roseboom T, de Rooij S, Painter R (2006): The Dutch famine and its long-term consequences for adult health. Early Hum Dev 82:485–491.
3. de Rooij SR, Wouters H, Yonker JE, Painter RC, Roseboom TJ (2010): Prenatal undernutrition and cognitive function in late adulthood. Proc Natl Acad Sci USA 107:16881–16886.
4. Schulz LC (2010): The Dutch Hunger Winter and the developmental origins of health and disease. Proc Natl Acad Sci USA 107:16757–16758.
5. St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F, et al. (2005): Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959–1961. JAMA 294:557–562.
6. Romo A, Carceller R, Tobajas J (2009): Intrauterine growth retardation (IUGR): Epidemiology and etiology. Pediatr Endocrinol Rev 6(suppl 3):332–336.
7. Castanys-Muñoz E, Kennedy K, Castañeda-Gutiérrez E, Forsyth S, Godfrey KM, Koletzko B, et al. (2017): Systematic review indicates postnatal growth in term infants born small-for-gestational-age being associated with later neurocognitive and metabolic outcomes. Acta Paediatr 106:1230–1238.
8. O'Donnell KJ, Meaney MJ (2017): Fetal origins of mental health: The developmental origins of health and disease hypothesis. Am J Psychiatry 174:319–328.
9. Jansson T, Powell TL (2006): IFPA 2005 award in placentology lecture. Human placental transport in altered fetal growth: Does the placenta function as a nutrient sensor? – A review. Placenta 27(suppl A):S91–S97.
10. Pantham P, Rosario FJ, Weintraub ST, Nathanielsz PW, Powell TL, Li C, Jansson T (2016): Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in maternal nutrient restricted baboons. Biol Reprod 95:98.
11. Rosario FJ, Kramer A, Li C, Galan HL, Powell TL, Nathanielsz PW, Jansson T (2021): Reduction of in vivo placental amino acid transport precedes the development of intrauterine growth restriction in the non-human primate. Nutrients 13:2892.
12. Bajoria R, Sooranna SR, Ward S, D'Souza S, Hancock M (2001): Placental transport rather than maternal concentration of amino acids regulates fetal growth in monochorionic twins: Implications for fetal origin hypothesis. Am J Obstet Gynecol 185:1239–1246.
13. Erokhin V, Diao L, Gao T, Andrei J-V, Ivolga A, Zong Y (2021): The supply of calories, proteins, and fats in low-income countries: A four-decade retrospective study. Int J Environ Res Public Health 18:7356.
14. Henchion M, Hayes M, Mullen AM, Fenelon M, Tiwari B (2017): Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. Foods 6:1–21.
15. Stephens TV, Payne M, Ball RO, Pencharz PB, Elango R (2015): Protein requirements of healthy pregnant women during early and late gestation are higher than current recommendations. J Nutr 145:73–78.
16. Elango R, Ball RO (2016): Protein and amino acid requirements during pregnancy. Adv Nutr 7:839S–844S.
17. Murphy MM, Higgins KA, Bi X, Barraj LM (2021): Adequacy and sources of protein intake among pregnant women in the United States, NHANES 2003–2012. Nutrients 13:1–13.
18. European Union Horizon 2020 research and innovation programme (No 862957) (2021): What Consumers Want: A Survey on European Consumer Attitudes Towards Plant-Based Food Available at: https://smartproteinproject.eu/consumer-attitudes-plant-based-food-report/. Accessed April 18, 2023.
19. Manta-Vogli PD, Schulpis KH, Dotsikas Y, Loukas YL (2020): The significant role of amino acids during pregnancy: Nutritional support. J Matern Fetal Neonatal Med 33:334–340.
20. Mariotti F, Gardner CD (2019): Dietary protein and amino acids in vegetarian diets-A review. Nutrients 11:2661.
21. Schmidt JA, Rinaldi S, Scalbert A, Ferrari P, Achaintre D, Gunter MJ, et al. (2016): Plasma concentrations and intakes of amino acids in male meat-eaters, fish-eaters, vegetarians and vegans: A cross-sectional analysis in the EPIC-Oxford cohort. Eur J Clin Nutr 70:306–312.
22. Camilleri GM, Verger EO, Huneau J-F, Carpentier F, Dubuisson C, Mariotti F (2013): Plant and animal protein intakes are differently associated with nutrient adequacy of the diet of French adults. J Nutr 143:1466–1473.
23. Ryckman KK, Donovan BM, Fleener DK, Bedell B, Borowski KS (2016): Pregnancy-related changes of amino acid and acylcarnitine concentrations: The impact of obesity. AJP Rep 6:e329–e336.
24. Verger EO, Aron-Wisnewsky J, Dao MC, Kayser BD, Oppert J-M, Bouillot J-L, et al. (2016): Micronutrient and Protein Deficiencies after Gastric Bypass and Sleeve Gastrectomy: A 1-year Follow-up. Obes Surg 26:785–796.
25. Falcone V, Stopp T, Feichtinger M, Kiss H, Eppel W, Husslein PW, et al. (2018): Pregnancy after bariatric surgery: A narrative literature review and discussion of impact on pregnancy management and outcome. BMC Pregnancy Childbirth 18:507.
26. Gómez-Cantarino S, Agulló-Ortuño MT, de Dios-Aguado M, Ugarte-Gurrutxaga MI, Bouzas-Mosquera C (2020): Prevalence of hypoproteinemia and hypoalbuminemia in pregnant women from three different socioeconomic populations. Int J Environ Res Public Health 17:1–10.
27. Scher MS (2021): "The First Thousand Days" define a fetal/neonatal neurology program. Front Pediatr 9:683138.
28. Kolb B, Gibb R (2011): Brain plasticity and behaviour in the developing brain. J Can Acad Child Adolesc Psychiatry 20:265–276.
29. Bowers ME, Yehuda R (2016): Intergenerational transmission of stress in humans. Neuropsychopharmacology 41:232–244.
30. Faa G, Manchia M, Pintus R, Gerosa C, Marcialis MA, Fanos V (2016): Fetal programming of neuropsychiatric disorders. Birth Defects Res C Embryo Today 108:207–223.

31. Stephens BE, Walden RV, Gargus RA, Tucker R, McKinley L, Mance M, et al. (2009): First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123:1337–1343.

32. Rozé J-C, Morel B, Lapillonne A, Marret S, Guellec I, Darmaun D, et al. (2021): Association between early amino acid intake and full-scale IQ at Age 5 years among infants born at less than 30 weeks' gestation. JAMA Netw Open 4:e2135452.

33. Eichenauer H, Ehlert U (2023): The association between prenatal famine, DNA methylation and mental disorders: A systematic review and meta-analysis. Clin Epigenetics 15:152.

34. McMullen S, Mostyn A (2009): Animal models for the study of the developmental origins of health and disease. Proc Nutr Soc 68:306–320.

35. Bautista CJ, Bautista RJ, Montaño S, Reyes-Castro LA, Rodriguez-Peña ON, Ibáñez CA, et al. (2019): Effects of maternal protein restriction during pregnancy and lactation on milk composition and offspring development. Br J Nutr 122:141–151.

36. Bhasin KKS, van Nas A, Martin LJ, Davis RC, Devaskar SU, Lusis AJ (2009): Maternal low-protein diet or hypercholesterolemia reduces circulating essential amino acids and leads to intrauterine growth restriction. Diabetes 58:559–566.

37. Rees WD, Hay SM, Buchan V, Antipatis C, Palmer RM (1999): The effects of maternal protein restriction on the growth of the rat fetus and its amino acid supply. Br J Nutr 81:243–250.

38. Malandro MS, Beveridge MJ, Kilberg MS, Novak DA (1996): Effect of low-protein diet-induced intrauterine growth retardation on rat placental amino acid transport. Am J Physiol 271:C295–C303.

39. Jansson N, Pettersson J, Haafiz A, Ericsson A, Palmberg I, Tranberg M, et al. (2006): Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet. J Physiol 576:935–946.

40. Gaccioli F, Lager S, Powell TL, Jansson T (2013): Placental transport in response to altered maternal nutrition. J Dev Orig Health Dis 4:101–115.

41. Vaughan OR, Rosario FJ, Powell TL, Jansson T (2017): Regulation of placental amino acid transport and fetal growth. Prog Mol Biol Transl Sci 145:217–251.

42. Alexandre-Gouabau M-C, Courant F, Le Gall G, Moyon T, Darmaun D, Parnet P, et al. (2011): Offspring metabolomic response to maternal protein restriction in a rat model of intrauterine growth restriction (IUGR). J Proteome Res 10:3292–3302.

43. Alexandre-Gouabau M-CF, Bailly E, Moyon TL, Grit IC, Coupé B, Le Drean G, et al. (2012): Postnatal growth velocity modulates alterations of proteins involved in metabolism and neuronal plasticity in neonatal hypothalamus in rats born with intrauterine growth restriction. J Nutr Biochem 23:140–152.

44. Martin Agnoux A, El Ghaziri A, Moyon T, Pagniez A, David A, Simard G, et al. (2018): Maternal protein restriction during lactation induces early and lasting plasma metabolomic and hepatic lipidomic signatures of the offspring in a rodent programming model. J Nutr Biochem 55:124–141.

45. Torres N, Bautista CJ, Tovar AR, Ordáz G, Rodríguez-Cruz M, Ortiz V, et al. (2010): Protein restriction during pregnancy affects maternal liver lipid metabolism and fetal brain lipid composition in the rat. Am J Physiol Endocrinol Metab 298:E270–E277.

46. Reyes-Castro LA, Rodriguez JS, Charco R, Bautista CJ, Larrea F, Nathanielsz PW, Zambrano E (2012): Maternal protein restriction in the rat during pregnancy and/or lactation alters cognitive and anxiety behaviors of female offspring. Int J Dev Neurosci 30:39–45.

47. Reyes-Castro LA, Rodriguez JS, Rodríguez-González GL, Chavira R, Bautista CJ, McDonald TJ, et al. (2012): Pre- and/or postnatal protein restriction developmentally programs affect and risk assessment behaviors in adult male rats. Behav Brain Res 227:324–329.

48. de Godoy MA, de Souza AS, Lobo MA, Sampaio OVK, Moraes L, Baldanza MR, et al. (2013): Effects of protein restriction during gestation and lactation on cell proliferation in the hippocampus and subventricular zone: Functional implications. Protein restriction alters hippocampal/SVZ cell proliferation. Brain Res 1496:10–27.

49. Crossland RF, Balasa A, Ramakrishnan R, Mahadevan SK, Fiorotto ML, Van den Veyver IB (2017): Chronic maternal low-protein diet in mice affects anxiety, night-time energy expenditure and sleep patterns, but not circadian rhythm in male offspring. PLoS One 12:e0170127.

50. Belluscio LM, Berardino BG, Ferroni NM, Ceruti JM, Cánepa ET (2014): Early protein malnutrition negatively impacts physical growth and neurological reflexes and evokes anxiety and depressive-like behaviors. Physiol Behav 129:237–254.

51. Pillay N, Rimbach R, Rymer T (2016): Pre- and postnatal dietary protein deficiency influences anxiety, memory and social behaviour in the African striped mouse Rhabdomys dilectus chakae. Physiol Behav 161:38–46.

52. Nätt D, Barchiesi R, Murad J, Feng J, Nestler EJ, Champagne FA, Thorsell A (2017): Perinatal malnutrition leads to sexually dimorphic behavioral responses with associated epigenetic changes in the mouse brain. Sci Rep 7:11082.

53. Raglan GB, Schmidt LA, Schulkin J (2017): The role of glucocorticoids and corticotropin-releasing hormone regulation on anxiety symptoms and response to treatment. Endocr Connect 6:R1–R7.

54. Torres DB, Lopes A, Rodrigues AJ, Lopes MG, Ventura-Silva AP, Sousa N, et al. (2023): Gestational protein restriction alters early amygdala neurochemistry in male offspring. Nutr Neurosci 26:1103–1119.

55. de Rooij SR, Painter RC, Phillips DIW, Osmond C, Tanck MWT, Bossuyt PMM, Roseboom TJ (2006): Cortisol responses to psychological stress in adults after prenatal exposure to the Dutch famine. Psychoneuroendocrinology 31:1257–1265.

56. de Rooij SR, Painter RC, Phillips DI, Räikkönen K, Schene AH, Roseboom TJ (2011): Self-reported depression and anxiety after prenatal famine exposure: Mediation by cardio-metabolic pathology? J Dev Orig Health Dis 2:136–143.

57. Fitzallen GC, Sagar YK, Taylor HG, Bora S (2021): Anxiety and depressive disorders in children born preterm: A meta-analysis. J Dev Behav Pediatr 42:154–162.

58. Avery SN, Clauss JA, Blackford JU (2016): The human BNST: Functional role in anxiety and addiction. Neuropsychopharmacology 41:126–141.

59. Ye W, Pitlock MD, Javors MA, Thompson BJ, Lechleiter JD, Hensler JG (2018): The long-term effect of maternal dietary protein restriction on 5-HT1A receptor function and behavioral responses to stress in adulthood. Behav Brain Res 349:116–124.

60. Morgane PJ, Austin-LaFrance R, Bronzino J, Tonkiss J, Díaz-Cintra S, Cintra L, et al. (1993): Prenatal malnutrition and development of the brain. Neurosci Biobehav Rev 17:91–128.

61. Blatt GJ, Chen JC, Rosene DL, Volicer L, Galler JR (1994): Prenatal protein malnutrition effects on the serotonergic system in the hippocampal formation: An immunocytochemical, ligand binding, and neurochemical study. Brain Res Bull 34:507–518.

62. Vucetic Z, Totoki K, Schoch H, Whitaker KW, Hill-Smith T, Lucki I, Reyes TM (2010): Early life protein restriction alters dopamine circuitry. Neuroscience 168:359–370.

63. Coviello C, Keunen K, Kersbergen KJ, Groenendaal F, Leemans A, Peels B, et al. (2018): Effects of early nutrition and growth on brain volumes, white matter microstructure, and neurodevelopmental outcome in preterm newborns. Pediatr Res 83:102–110.

64. Franke K, Van den Bergh BRH, de Rooij SR, Kroegel N, Nathanielsz PW, Rakers F, et al. (2020): Effects of maternal stress and nutrient restriction during gestation on offspring neuroanatomy in humans. Neurosci Biobehav Rev 117:5–25.

65. Yao B, Christian KM, He C, Jin P, Ming G-L, Song H (2016): Epigenetic mechanisms in neurogenesis. Nat Rev Neurosci 17:537–549.

66. Dehay C, Kennedy H (2007): Cell-cycle control and cortical development. Nat Rev Neurosci 8:438–450.

67. Wang L, Xu R-J (2007): The effects of perinatal protein malnutrition on spatial learning and memory behaviour and brain-derived neurotrophic factor concentration in the brain tissue in young rats. Asia Pac J Clin Nutr 16(suppl 1):467–472.

68. Lister JP, Blatt GJ, DeBassio WA, Kemper TL, Tonkiss J, Galler JR, Rosene DL (2005): Effect of prenatal protein malnutrition on numbers of neurons in the principal cell layers of the adult rat hippocampal formation. Hippocampus 15:393–403.

69. Pérez-García G, Guzmán-Quevedo O, Da Silva Aragão R, Bolaños-Jiménez F (2016): Early malnutrition results in long-lasting impairments

in pattern-separation for overlapping novel object and novel location memories and reduced hippocampal neurogenesis. Sci Rep 6:21275.

70. Fesser EA, Gianatiempo O, Berardino BG, Alberca CD, Urrutia L, Falasco G, et al. (2022): Impaired social cognition caused by perinatal protein malnutrition evokes neurodevelopmental disorder symptoms and is intergenerationally transmitted. Exp Neurol 347:113911.

71. Neitzke U, Harder T, Plagemann A (2011): Intrauterine growth restriction and developmental programming of the metabolic syndrome: A critical appraisal. Microcirculation 18:304–311.

72. Rinaudo P, Wang E (2012): Fetal programming and metabolic syndrome. Annu Rev Physiol 74:107–130.

73. Croizier S, Bouret SG (2022): Molecular control of the development of hypothalamic neurons involved in metabolic regulation. J Chem Neuroanat 123:102117.

74. Padilla SL, Carmody JS, Zeltser LM (2010): Pomc-expressing progenitors give rise to antagonistic neuronal populations in hypothalamic feeding circuits. Nat Med 16:403–405.

75. Blanco N, Fernández-García JM, Carrillo B, Ballesta A, García-Úbeda R, Collado P, Pinos H (2022): Prenatal low-protein and low-calorie diets differentially alter arcuate nucleus morphology in newborn male rats. Front Neuroanat 16:896732.

76. Desai M, Han G, Li T, Ross MG (2019): Programmed epigenetic DNA methylation-mediated reduced neuroprogenitor cell proliferation and differentiation in small-for-gestational-age offspring. Neuroscience 412:60–71.

77. Plagemann A, Harder T, Rake A, Melchior K, Rohde W, Dörner G (2000): Hypothalamic nuclei are malformed in weanling offspring of low protein malnourished rat dams. J Nutr 130:2582–2589.

78. Coupé B, Amarger V, Grit I, Benani A, Parnet P (2010): Nutritional programming affects hypothalamic organization and early response to leptin. Endocrinology 151:702–713.

79. Desai M, Li T, Ross MG (2011): Hypothalamic neurosphere progenitor cells in low birth-weight rat newborns: Neurotrophic effects of leptin and insulin. Brain Res 1378:29–42.

80. de Oliveira JC, Gomes RM, Miranda RA, Barella LF, Malta A, Martins IP, et al. (2016): Protein restriction during the last third of pregnancy Malprograms the neuroendocrine axes to induce metabolic syndrome in adult male rat offspring. Endocrinology 157:1799–1812.

81. Moullé VS, Frapin M, Amarger V, Parnet P (2023): Maternal protein restriction in rats alters postnatal growth and brain lipid sensing in female offspring. Nutrients 15:463.

82. Terroni PL, Anthony FW, Hanson MA, Cagampang FRA (2005): Expression of agouti-related peptide, neuropeptide Y, pro-opiomelanocortin and the leptin receptor isoforms in fetal mouse brain from pregnant dams on a protein-restricted diet. Brain Res Mol Brain Res 140:111–115.

83. Frapin M, Guignard S, Meistermann D, Grit I, Moullé VS, Paillé V, et al. (2020): Maternal protein restriction in rats alters the expression of genes involved in mitochondrial metabolism and epitranscriptomics in fetal hypothalamus. Nutrients 12:E1464.

84. Brown AS, Susser ES, Lin SP, Neugebauer R, Gorman JM (1995): Increased risk of affective disorders in males after second trimester prenatal exposure to the Dutch hunger winter of 1944–45. Br J Psychiatry 166:601–606.

85. de Rooij SR, Caan MWA, Swaab DF, Nederveen AJ, Majoie CB, Schwab M, et al. (2016): Prenatal famine exposure has sex-specific effects on brain size. Brain 139:2136–2142.

86. Gressens P, Muaku SM, Besse L, Nsegbe E, Gallego J, Delpech B, et al. (1997): Maternal protein restriction early in rat pregnancy alters brain development in the progeny. Brain Res Dev Brain Res 103:21–35.

87. Watkins AJ, Ursell E, Panton R, Papenbrock T, Hollis L, Cunningham C, et al. (2008): Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. Biol Reprod 78:299–306.

88. Mueller BR, Bale TL (2008): Sex-specific programming of offspring emotionality after stress early in pregnancy. J Neurosci 28:9055–9065.

89. Green CL, Pak HH, Richardson NE, Flores V, Yu D, Tomasiewicz JL, et al. (2022): Sex and genetic background define the metabolic, physiologic, and molecular response to protein restriction. Cell Metab 34:209–226.e5.

90. Toivonen KI, Lacroix E, Flynn M, Ronksley PE, Oinonen KA, Metcalfe A, Campbell TS (2018): Folic acid supplementation during the preconception period: A systematic review and meta-analysis. Prev Med 114:1–17.

91. Gould JM, Smith PJ, Airey CJ, Mort EJ, Airey LE, Warricker FDM, et al. (2018): Mouse maternal protein restriction during preimplantation alone permanently alters brain neuron proportion and adult short-term memory. Proc Natl Acad Sci USA 115:E7398–E7407.

92. Khurana P, Cox A, Islam B, Eckert JJ, Willaime-Morawek S, Gould JM, et al. (2023): Maternal undernutrition induces cell signalling and metabolic dysfunction in undifferentiated mouse embryonic stem cells. Stem Cell Rev Rep 19:767–783.

93. Eckert JJ, Porter R, Watkins AJ, Burt E, Brooks S, Leese HJ, et al. (2012): Metabolic induction and early responses of mouse blastocyst developmental programming following maternal low protein diet affecting life-long health. PLoS One 7:e52791.

94. Berger SL, Sassone-Corsi P (2016): Metabolic signaling to chromatin. Cold Spring Harb Perspect Biol 8:a019463.

95. Lopes A FC (2020): Mitochondrial metabolism and DNA methylation: A review of the interaction between two genomes. Clin Epigenetics 12:182.

96. Bekdash RA (2023): Methyl donors, epigenetic alterations, and brain health: Understanding the connection. Int J Mol Sci 24:2346.

97. Ji Y, Wu Z, Dai Z, Sun K, Wang J, Wu G (2016): Nutritional epigenetics with a focus on amino acids: Implications for the development and treatment of metabolic syndrome. J Nutr Biochem 27:1–8.

98. Amarger V, Lecouillard A, Ancellet L, Grit I, Castellano B, Hulin P, Parnet P (2014): Protein content and methyl donors in maternal diet interact to influence the proliferation rate and cell fate of neural stem cells in rat hippocampus. Nutrients 6:4200–4217.

99. Marwarha G, Claycombe-Larson K, Schommer J, Ghribi O (2017): Maternal low-protein diet decreases brain-derived neurotrophic factor expression in the brains of the neonatal rat offspring. J Nutr Biochem 45:54–66.

100. Matsumura Y, Wei F-Y, Sakai J (2023): Epitranscriptomics in metabolic disease. Nat Metab 5:370–384.

101. Peter CJ, Fischer LK, Kundakovic M, Garg P, Jakovcevski M, Dincer A, et al. (2016): DNA methylation signatures of early childhood malnutrition associated with impairments in attention and cognition. Biol Psychiatry 80:765–774.

102. Mulligan CJ, D'Errico NC, Stees J, Hughes DA (2012): Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. Epigenetics 7:853–857.

103. Koo B, Lee K-H, Ming G-L, Yoon K-J, Song H (2023): Setting the clock of neural progenitor cells during mammalian corticogenesis. Semin Cell Dev Biol 142:43–53.

104. Takahara T, Amemiya Y, Sugiyama R, Maki M, Shibata H (2020): Amino acid-dependent control of mTORC1 signaling: A variety of regulatory modes. J Biomed Sci 27:87.

105. LiCausi F, Hartman NW (2018): Role of mTOR complexes in neurogenesis. Int J Mol Sci 19:1544.

106. Bifari F, Dolci S, Bottani E, Pino A, Di Chio M, Zorzin S, et al. (2020): Complete neural stem cell (NSC) neuronal differentiation requires a branched chain amino acids-induced persistent metabolic shift towards energy metabolism. Pharmacol Res 158:104863.

107. Zheng X, Boyer L, Jin M, Mertens J, Kim Y, Ma L, et al. (2016): Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. eLife 5: e13374.

108. Knaus LS, Basilico B, Malzl D, Gerykova Bujalkova M, Smogavec M, Schwarz LA, et al. (2023): Large neutral amino acid levels tune perinatal neuronal excitability and survival. Cell 186:1950–1967.e25.

109. Tărlungeanu DC, Deliu E, Dotter CP, Kara M, Janiesch PC, Scalise M, et al. (2016): Impaired amino acid transport at the blood brain barrier is a cause of autism spectrum disorder. Cell 167:1481–1494.e18.

110. Müller J, Heuer H (2014): Expression pattern of thyroid hormone transporters in the postnatal mouse brain. Front Endocrinol (Lausanne) 5:92.

111. Cascio L, Chen C-F, Pauly R, Srikanth S, Jones K, Skinner CD, et al. (2020): Abnormalities in the genes that encode Large Amino Acid

Transporters increase the risk of autism spectrum disorder. Mol Genet Genomic Med 8:e1036.

112. Barbeito-Andrés J, Pezzuto P, Higa LM, Dias AA, Vasconcelos JM, Santos TMP, et al. (2020): Congenital Zika syndrome is associated with maternal protein malnutrition. Sci Adv 6:eaaw6284.

113. Allgäuer L, Cabungcal J-H, Yzydorczyk C, Do KQ, Dwir D (2023): Low protein-induced intrauterine growth restriction as a risk factor for schizophrenia phenotype in a rat model: Assessing the role of oxidative stress and neuroinflammation interaction. Transl Psychiatry 13:30.

114. Vacher C-M, Bonnin A, Mir IN, Penn AA (2023): Editorial: Advances and perspectives in neuroplacentology. Front Endocrinol (Lausanne) 14:1206072.

115. Cleal JK, Lofthouse EM, Sengers BG, Lewis RM (2018): A systems perspective on placental amino acid transport. J Physiol 596:5511–5522.

116. Tran N-T, Amarger V, Bourdon A, Misbert E, Grit I, Winer N, Darmaun D (2017): Maternal citrulline supplementation enhances placental function and fetal growth in a rat model of IUGR: Involvement of insulin-like growth factor 2 and angiogenic factors. J Matern Fetal Neonatal Med 30:1906–1911.

117. Bourdon A, Hannigsberg J, Misbert E, Tran TN, Amarger V, Ferchaud-Roucher V, et al. (2020): Maternal supplementation with citrulline or arginine during gestation impacts fetal amino acid availability in a model of intrauterine growth restriction (IUGR). Clin Nutr 39:3736–3743.

118. Bourdon A, Parnet P, Nowak C, Tran N-T, Winer N, Darmaun D (2016): L-citrulline supplementation enhances fetal growth and protein synthesis in rats with intrauterine growth restriction. J Nutr 146:532–541.

119. Berdenis van Berlekom A, Kübler R, Hoogeboom JW, Vonk D, Sluijs JA, Pasterkamp RJ, et al. (2022): Exposure to the amino acids histidine, lysine, and threonine reduces mTOR activity and affects neurodevelopment in a human cerebral organoid model. Nutrients 14:2175.

120. Xu J, Jakher Y, Ahrens-Nicklas RC (2020): Brain branched-chain amino acids in maple syrup urine disease: Implications for neurological disorders. Int J Mol Sci 21:1–18.

121. Burlina AP, Lachmann RH, Manara R, Cazzorla C, Celato A, van Spronsen FJ, Burlina A (2019): The neurological and psychological phenotype of adult patients with early-treated phenylketonuria: A systematic review. J Inherit Metab Dis 42:209–219.

122. Klapp A-L, Feil N, Risius A (2022): A global analysis of national dietary guidelines on plant-based diets and substitutions for animal-based foods. Curr Dev Nutr 6:nzac144.

123. Zeiss CJ (2021): Comparative milestones in rodent and human postnatal central nervous system development. Toxicol Pathol 49:1368–1373.

124. Al Zaid Siddiquee K, Turkson J (2008): STAT3 as a target for inducing apoptosis in solid and hematological tumors. Cell Res 18:254–267.

125. Wiegersma AM, Boots A, Roseboom TJ, de Rooij SR (2022): Prenatal exposure to the Dutch famine is associated with more self-perceived cognitive problems at 72 years of age. BMC Geriatr 22:176.

126. Hulshoff Pol HE, Hoek HW, Susser E, Brown AS, Dingemans A, Schnack HG, et al. (2000): Prenatal exposure to famine and brain morphology in schizophrenia. Am J Psychiatry 157:1170–1172.

127. Rong H, Lai X, Mahmoudi E, Fang H (2019): Early-life exposure to the Chinese famine and risk of cognitive decline. J Clin Med 8:484.

128. Li H, Barnhart HX, Stein AD, Martorell R (2003): Effects of early childhood supplementation on the educational achievement of women. Pediatrics 112:1156–1162.

129. Xie L, Antonow-Schlorke I, Schwab M, McDonald TJ, Nathanielsz PW, Li C (2013): The frontal cortex IGF system is down regulated in the term, intrauterine growth restricted fetal baboon. Growth Horm IGF Res 23:187–192.

130. Li C, Ramahi E, Nijland MJ, Choi J, Myers DA, Nathanielsz PW, McDonald TJ (2013): Up-regulation of the fetal baboon hypothalamo-pituitary-adrenal axis in intrauterine growth restriction: Coincidence with hypothalamic glucocorticoid receptor insensitivity and leptin receptor down-regulation. Endocrinology 154:2365–2373.

131. Li C, McDonald TJ, Wu G, Nijland MJ, Nathanielsz PW (2013): Intrauterine growth restriction alters term fetal baboon hypothalamic appetitive peptide balance. J Endocrinol 217:275–282.

132. Ye W, Xie L, Li C, Nathanielsz PW, Thompson BJ (2014): Impaired development of fetal serotonergic neurons in intrauterine growth restricted baboons. J Med Primatol 43:284–287.

133. Antonow-Schlorke I, Schwab M, Cox LA, Li C, Stuchlik K, Witte OW, et al. (2011): Vulnerability of the fetal primate brain to moderate reduction in maternal global nutrient availability. Proc Natl Acad Sci USA 108:3011–3016.

134. Rodriguez JS, Bartlett TQ, Keenan KE, Nathanielsz PW, Nijland MJ (2012): Sex-dependent cognitive performance in baboon offspring following maternal caloric restriction in pregnancy and lactation. Reprod Sci 19:493–504.

135. Abey NO, Ebuehi OAT, Imaga NOA (2019): Neurodevelopment and cognitive impairment in parents and progeny of perinatal dietary protein deficiency models. Front Neurosci 13:826.

136. Torres DB, Lopes A, Rodrigues AJ, Cerqueira JJ, Sousa N, Gontijo JAR, Boer PA (2018): Anxiety-like behavior and structural changes of the bed nucleus of the stria terminalis (BNST) in gestational protein-restricted male offspring. J Dev Orig Health Dis 9:536–543.

137. Lopes A, Torres DB, Rodrigues AJ, Cerqueira JJ, Pêgo JM, Sousa N, et al. (2013): Gestational protein restriction induces CA3 dendritic atrophy in dorsal hippocampal neurons but does not alter learning and memory performance in adult offspring. Int J Dev Neurosci 31:151–156.

138. Guest PC, Urday S, Ma D, Stelzhammer V, Harris LW, Amess B, et al. (2012): Proteomic analysis of the maternal protein restriction rat model for schizophrenia: Identification of translational changes in hormonal signaling pathways and glutamate neurotransmission. Proteomics 12:3580–3589.

139. Ranade SC, Sarfaraz Nawaz M, Kumar Rambtla P, Rose AJ, Gressens P, Mani S (2012): Early protein malnutrition disrupts cerebellar development and impairs motor coordination. Br J Nutr 107:1167–1175.

140. Lopes de Souza S, Orozco-Solis R, Grit I, Manhães de Castro R, Bolaños-Jiménez F (2008): Perinatal protein restriction reduces the inhibitory action of serotonin on food intake. Eur J Neurosci 27:1400–1408.

141. Orozco-Solís R, Matos RJB, Guzmán-Quevedo O, Lopes de Souza S, Bihouée A, Houlgatte R, et al. (2010): Nutritional programming in the rat is linked to long-lasting changes in nutrient sensing and energy homeostasis in the hypothalamus. PLoS One 5:e13537.

142. Díaz-Cintra S, González-Maciel A, Morales MA, Aguilar A, Cintra L, Prado-Alcalá RA (2007): Protein malnutrition differentially alters the number of glutamic acid decarboxylase-67 interneurons in dentate gyrus and CA1-3 subfields of the dorsal hippocampus. Exp Neurol 208:47–53.

143. Bennis-Taleb N, Remacle C, Hoet JJ, Reusens B (1999): A low-protein isocaloric diet during gestation affects brain development and alters permanently cerebral cortex blood vessels in rat offspring. J Nutr 129:1613–1619.