Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS

Physiology and Targeted Nutrition in Infants

ANNENBERG CENTER FOR HEALTH SCIENCES
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Learning Objectives

- Evaluate how targeted nutrition impacts physiological development
- Apply evidence-based nutrient research to disease risk and long-term development in preterm and term infants
- Recognize the role of nutrition in early brain development to improve postnatal outcomes

INTRODUCTION

Rigor and Reproducibility in Science Nutrition Research

1

Scientific rigor and credibility in the nutrition research landscape

Cynthia M Kroeger,[1] Cutberto Garza,[2] Christopher J Lynch,[3] Esther Myers,[4] Sylvia Rowe,[5] Barbara O Schneeman,[6] Arya M Sharma,[7] and David B Allison[1]

2

Best practices in nutrition science to earn and keep the public's trust

Cutberto Garza,[1] Patrick J Stover,[2] Sarah D Ohlhorst,[3] Martha S Field,[1] Robert Steinbrook,[4] Sylvia Rowe,[5] Catherine Woteki,[6] and Eric Campbell[7]

3

VIEWPOINT

The Challenge of Reforming Nutritional Epidemiologic Research

4

VIEWPOINT

The Need for Greater Rigor in Childhood Nutrition and Obesity Research

1. Kroeger CM, et al. Am J Clin Nutr. 2018;107(3):484-494. **2.** Garza C, et al. Am J Clin Nutr. 2019;109(1):225-243. **3.** Ioannidis JPA. JAMA. 2018;320(10):969-970. **4.** Wood AC, Wren JD, Allison DB. JAMA Pediatr. 2019.

Rigor and Reproducibility in Science Nutrition Research

Neonatal Nutrition: Unique Opportunity to Reveal Nutrient-Directed Effects on Human Physiology

The calls for increased rigor and reproducibility

- Should not detract from the evidence supporting the role of nutrition in improving survival and outcomes in preterm infants; a unique population that is captive, with limited exogenous exposures, and is in a rapidly developing window
- Should not lead to assumptions that adult physiological response to nutrition/nutrients is the same as the neonate
- Neonatal response to nutrition is unique and has a fundamental role in developmental physiology impacting health and disease risk
- It is imperative that all aspects of nutrition—practice and substrate delivery—be thoroughly investigated given the profound impact on health in the immediate and in the long term

Developmental Origins

- Developmental perspective on risk for adult disease
  - The "Barker Hypothesis" → "Fetal Origins" → DOHaD
- Early life events affect relevant long-term health outcomes
  - Cardiovascular
  - Metabolic
  - Immunologic and allergic
  - Cancer
  - **Mental Health**
- Potential biological mechanisms of the long-term neurobehavioral effects
- Clinical implications

DOHaD, Developmental Origins of Health and Disease.

Early Events and Later Outcomes

- Development is based on
  - Genetics
  - Epigenetics (experience-dependent influences)
- All organs, especially the brain, grow rapidly in late fetal/early neonatal period
  - Highly vulnerable to insults
  - Demonstrates its greatest plasticity/resilience and response to therapy

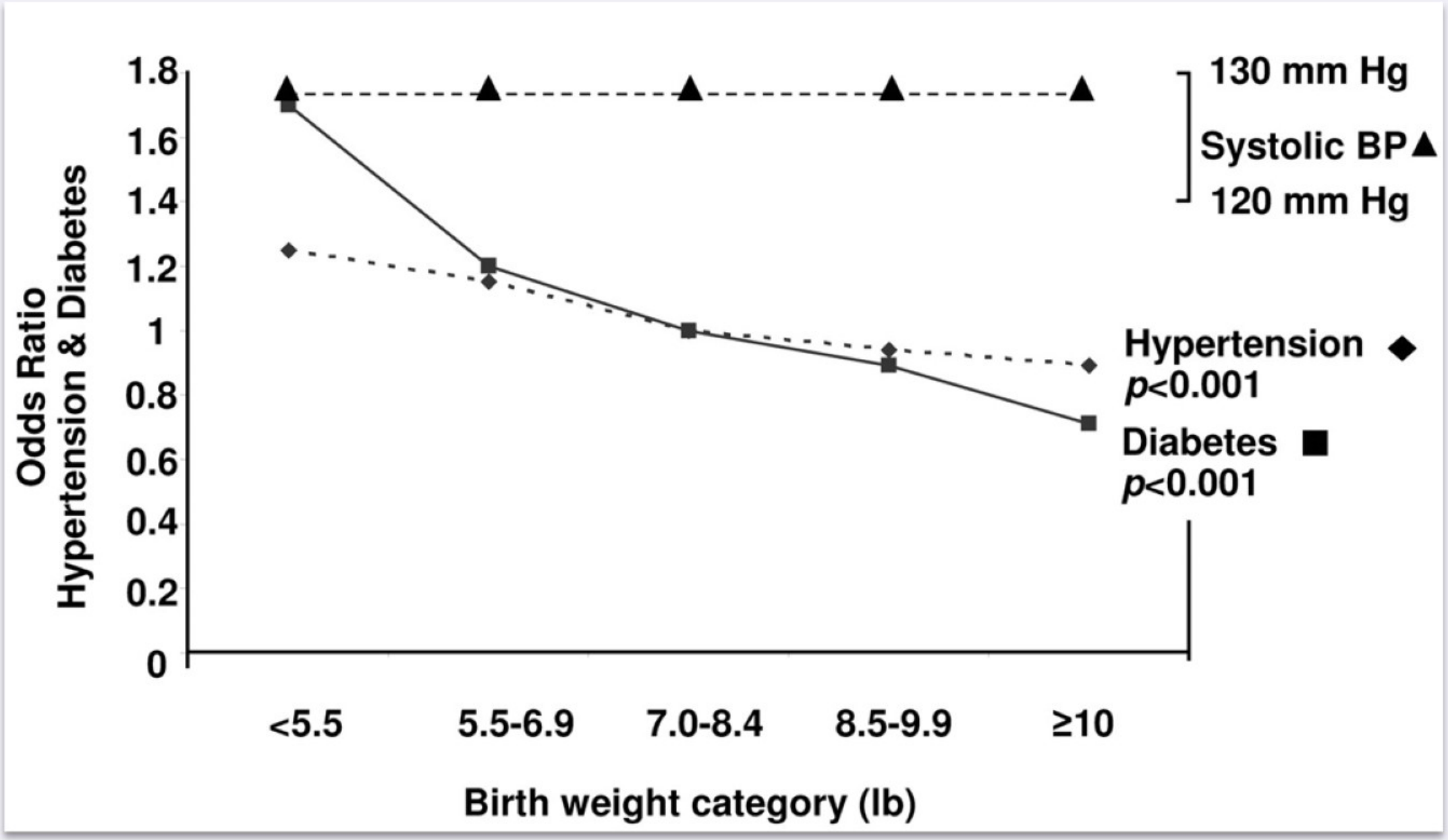
Fetal Programming

- "Programming" refers to epigenetic process
  - Early environmental stimuli (eg, **nutrition**) alter how genes are expressed throughout the lifetime
- Best described in fetal period with effect of prenatal nutrition → adult cardiovascular health (D. Barker)
- May also apply to postnatal nutrition in
  - Term and preterm infants
  - Adopted, orphaned children
  - Foster children
  - Children after severe illness
- Suggests vulnerable period based on postconceptional age irrespective of *in utero* vs *ex utero* (ie, no longer 'fetal')

What is the Barker Hypothesis?

- Studies by David Barker's group
  - Cohorts of adults in Britain with heart disease, diabetes mellitus, hypertension
  - Risk related, in part, to birth weight
  - Lower birth weight (ie, <7.0 lbs) increased risk
- Concept of altered metabolic set points *in utero*
  - Altered hypothalamic/pituitary/adrenal axis regulation (stress hormones)
  - Altered hepatic metabolism (especially carbohydrate handling)
  - Activation of proinflammatory cytokines

Barker DJ. *BMJ*. 1995;311:171-4.



Curhan GC, et al. *Circulation.* 1996;94:3246-50.
Gluckman PD, Hanson MA. *Science*. 2004;305:1733-1736. Used with permission from The American Association for the Advancement of Science.

NUTRITIONAL PROGRAMMING IN THE EARLY POSTNATAL PERIOD AND RISK OF NEONATAL MORBIDITIES

Nutrient-Directed Effects on Human Physiology

Adult Viewpoint

The potential to modulate the activity of the immune system by interventions with specific individual nutrients is termed *immunonutrition*.†

Infant Viewpoint

In the developing neonate, complex diets, medical practices, and individual nutrients have the potential to modulate the activity of the immune system, inflammation, and organogenesis—*nutritional programming*.

†Calder PC. *BMJ*. 2003; 327:117–118.

"...human infants actually remain helpless longer than infants of any other species and, ...must also go through a distinct period of gestation outside of the womb."

"This period of exterior gestation needs to be respected not just as a sentimental matter, but as one that has a profound and major impact on an infant's physical, emotional, and psychological development."

–Elizabeth Antunovic

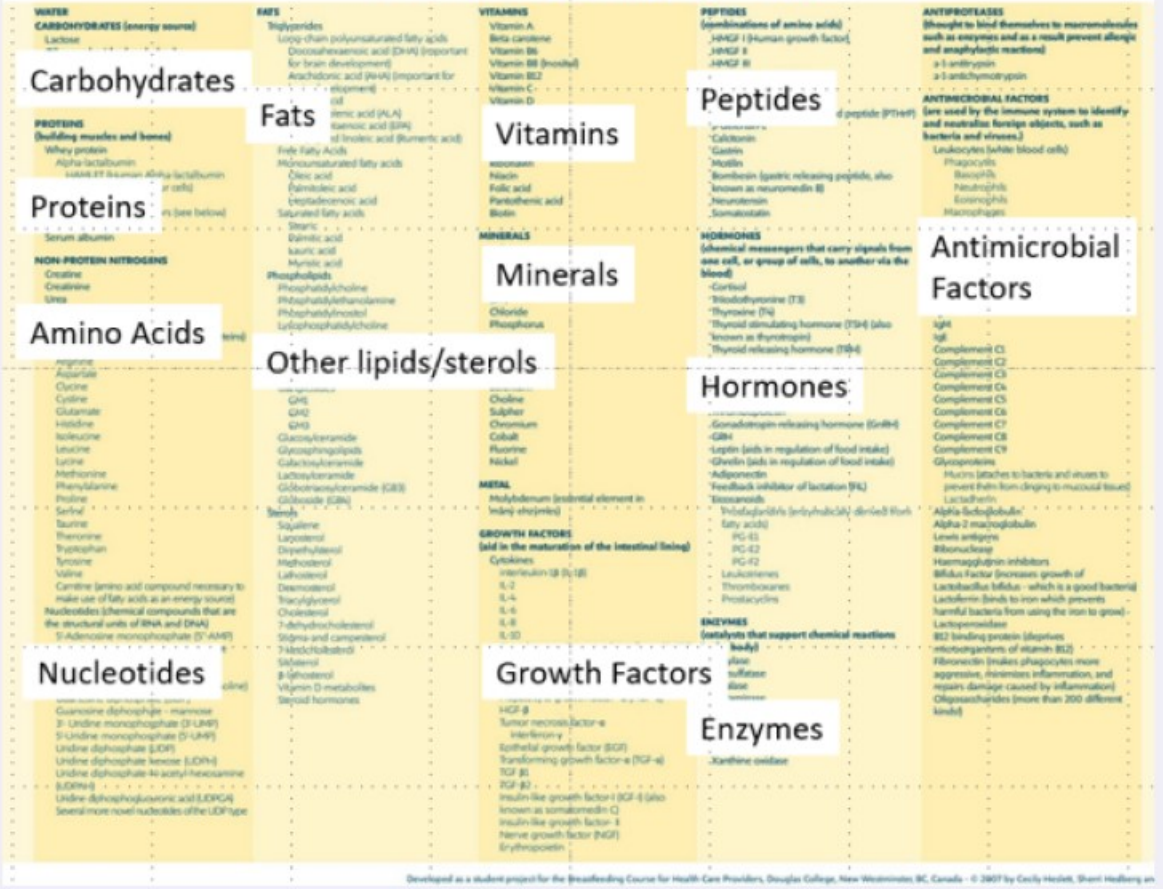
Antunovic E. The Second Nine Months: Exterogestation and the Need to be Held. Boba. August 2018.
http://www.boba.com/the-second-nine-months
Photo Credit: kipgodi/Bigstock

In Utero to *Ex Utero* Transition

Amniotic Fluid

Hormones	growth hormone, gastrin-releasing peptide, prolactin
Trophic or growth factors	epidermal growth factor, transforming growth factor-alpha, transforming growth factor beta-1; insulin-like growth factor I; erythropoietin, granulocyte colony-stimulating factor; hepatocyte growth factor, vasoactive endothelial growth factor
Nutrients and other proteins	water, electrolytes, carbohydrates, amino acids, lipids, albumin, serotransferrin, ceruloplasmin, alpha-fetoprotein, vitamin d-binding protein; apolipoprotein a1
Modulators of coagulation	antithrombin III, plasminogen
Modulators of immunity and inflammation	immunoglobulins, interleukins, complement, a-defensins, lactoferrin, lysozyme, calprotectin, cathelicidin, alpha1-antitrypsin, alpha1-microglobulin
Cell growth and differentiation	fibronectin; periostin; TGF-beta induced protein ig-h3 precursor; polyamines
Microbes	*?*

Breast Milk – Complex Matrix of Vital Immunonutrients



Modified from Cho CK, et al. *Mol Cell Proteomics*. 2007;6:1406-15.
Modified from image developed as a student project for the Breastfeeding Course for Health Care Providers, Douglas College, New Westminster, BC, Canada © 2007 by Cecily Heslett, Sherri Hedberg and Haley Rumble.

Mom's milk (nutrition) is critical in this period of *exterior gestation.*

Even more true for our preterm infants who still have to complete their first gestation!

Photo Credits: (Top) Romanova Anna/Bigstock, (Bottom) Metin Kiyak/Getty Images

During *exterior gestation* Mom's milk/nutrition provides necessary bioactive components to **protect** the infant from adverse environmental influences (as the infant is not ready to protect itself) while continuing the biological signaling for optimal organ **development** and ultimately healthy **independence**.

General Nutritional Delivery and Neonatal Outcomes

Early Nutrition Mediates the Influence of Severity of Illness on Extremely Low-Birth-Weight Infants[1]

- First 7 days, OR of NEC, late-onset sepsis, BPD, and NDI decreased by ~2% for each 1 kcal/ kg/d of total energy intake

First-Week Protein and Energy Intakes Are Associated With 18-Month Developmental Outcomes in Extremely Low-Birth-Weight Infants[2]

- An increase of 42 kJ (10 kcal)/kg per day independently associated with a ~5-point increase in MDI
- An increase of 1 g/kg per day of protein independently associated with a ~8-point increase in MDI

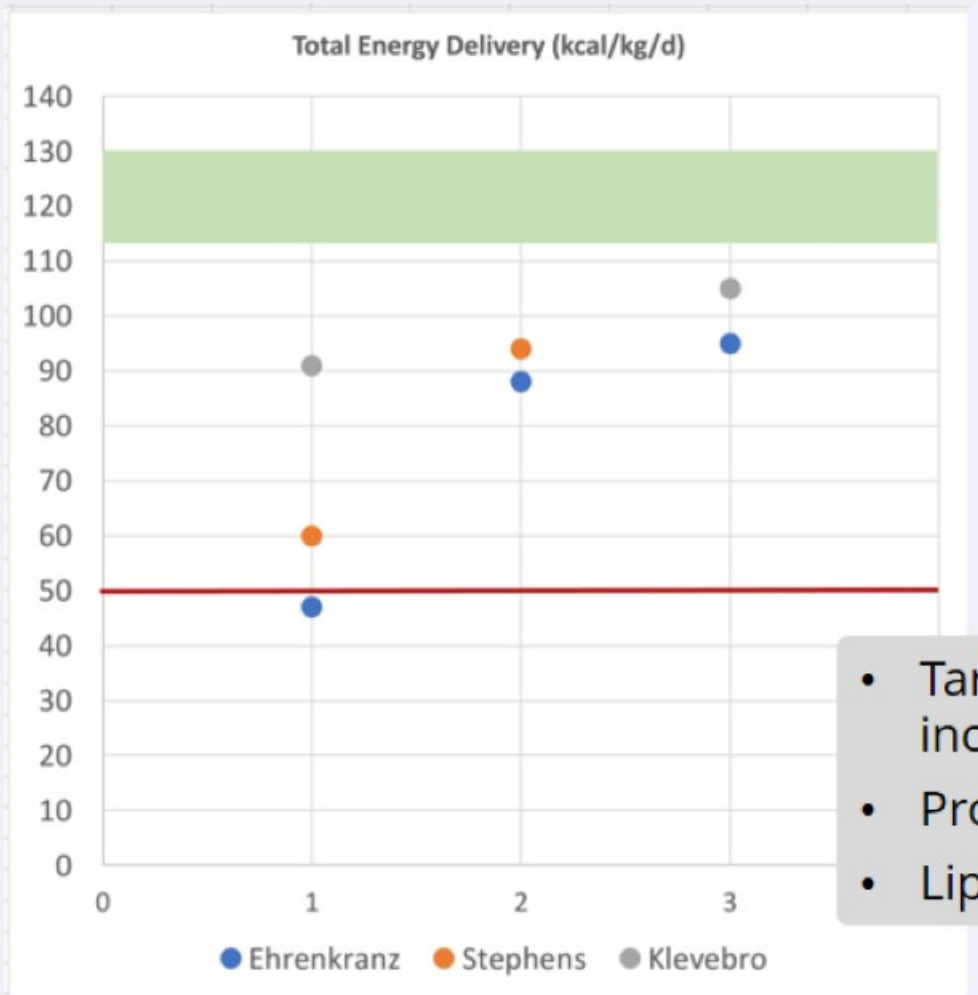
Early Energy and Protein Intakes and Associations With Growth, BPD, and ROP in Extremely Preterm Infants[3]

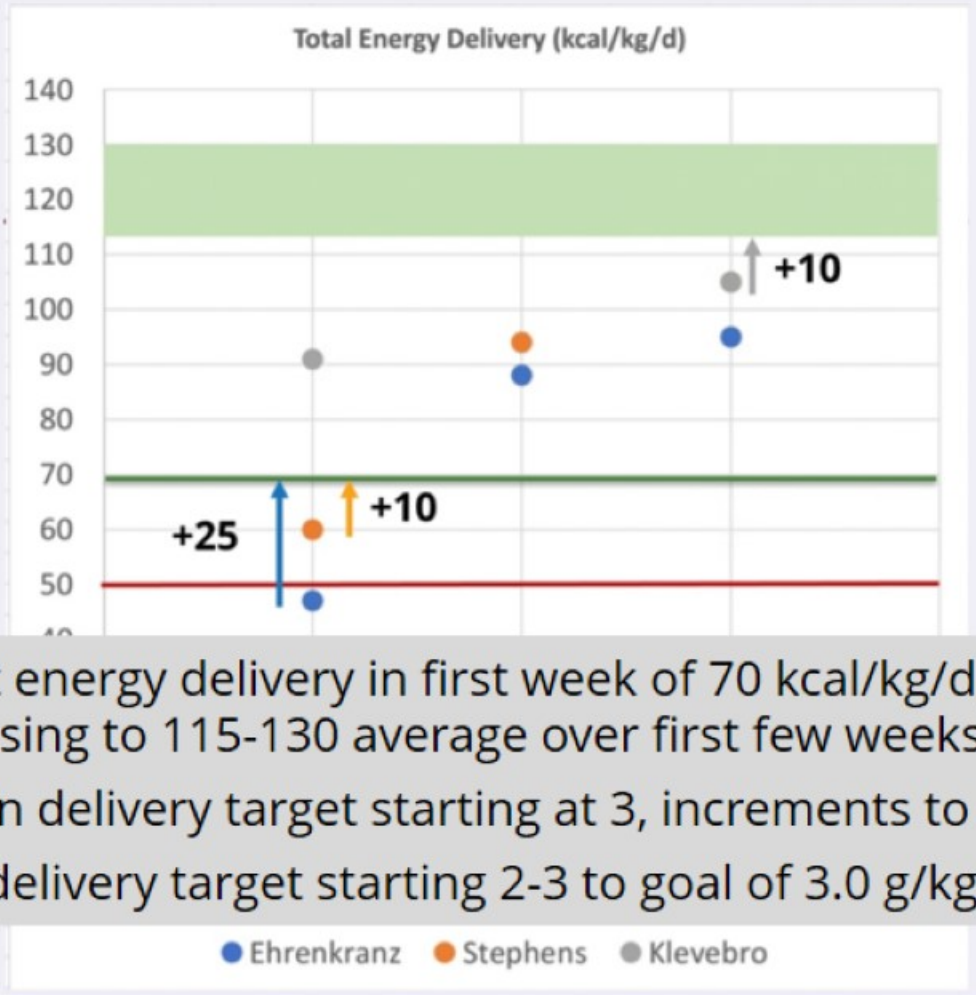
- Every 10 kcal/kg/d associated with 0.08 higher weight SD score
- Between d7-27, every 10 kcal/kg/d reduced risk of BPD of 9% and any grade of ROP of 6%
- Interaction MV, energy, protein: mean energy intake of 120 kcal/kg/d, every 0.5 g/kg/d reduced risk of BPD by 25%

BPD, bronchopulmonary dysplasia; MDI, mental developmental index; NDI, neurodevelopmental impairment; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity.

1. Ehrenkranz RA, et al. *Pediatr Res.* 2011;69:522-9.
2. Stephens BE, et al. *Pediatrics.* 2009;123:1337-43.
3. Klevebro S, et al. *Clin Nutr.* 2018. pii: S0261-5614:30197-3.

General Nutritional Delivery and Neonatal Outcomes





- Target energy delivery in first week of 70 kcal/kg/d, increasing to 115-130 average over first few weeks
- Protein delivery target starting at 3, increments to 4 g/kg/d
- Lipid delivery target starting 2-3 to goal of 3.0 g/kg/d

Koletzko, Poindexter, and Uauy. Nutritional Care of Preterm Infants: Scientific Basis and Practical Guidelines. S. Karger AG, 2014

General Nutritional Delivery and Neonatal Outcomes *(continued)*

Day	Lipids	Protein	Carbs	Rate - ml/k/d	Lipids	Protein	Carbs	Total=
1	2	3	10	100	18	12	40	70
	2.5	3	10	100	22.5	12	40	74.5
	3	3	10	100	27	12	40	79
2	2	3	10	120	18	12	48	78
	2.5	3	10	120	22.5	12	48	82.5
	3	3	10	120	27	12	48	87
3	2	3	10	140	18	12	56	86
	2.5	3	10	140	22.5	12	56	90.5
	3	3	10	140	27	12	56	95

Koletzko B, Poindexter B, Uauy R (Editors). Nutritional Care of Preterm Infants: Scientific Basis and Practical Guidelines. Basel, Karger, 2014.

INTESTINAL TRACT:
The Largest Immune Organ and Defense Barrier

- Direct exposure to nutrients
- Largest immune organ and defense barrier
- Gut-systemic health axis

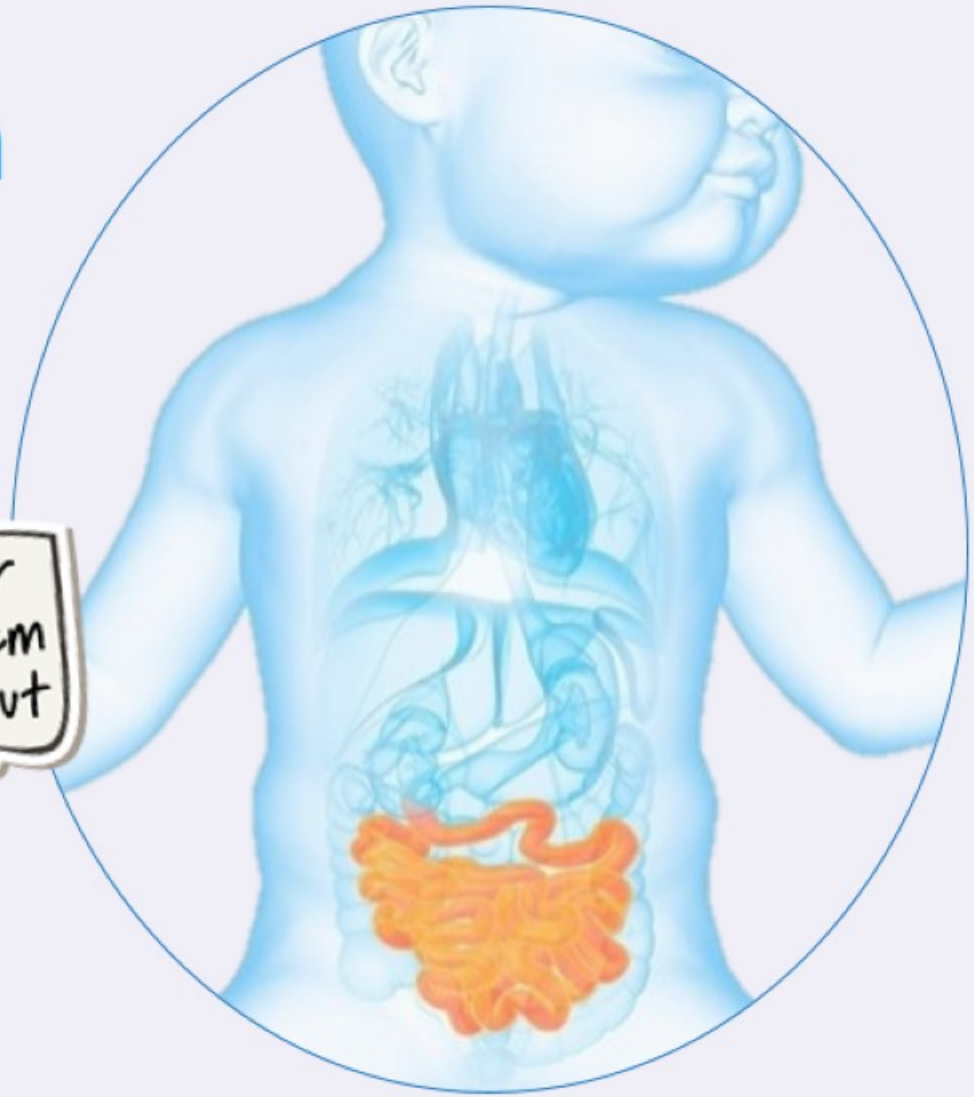
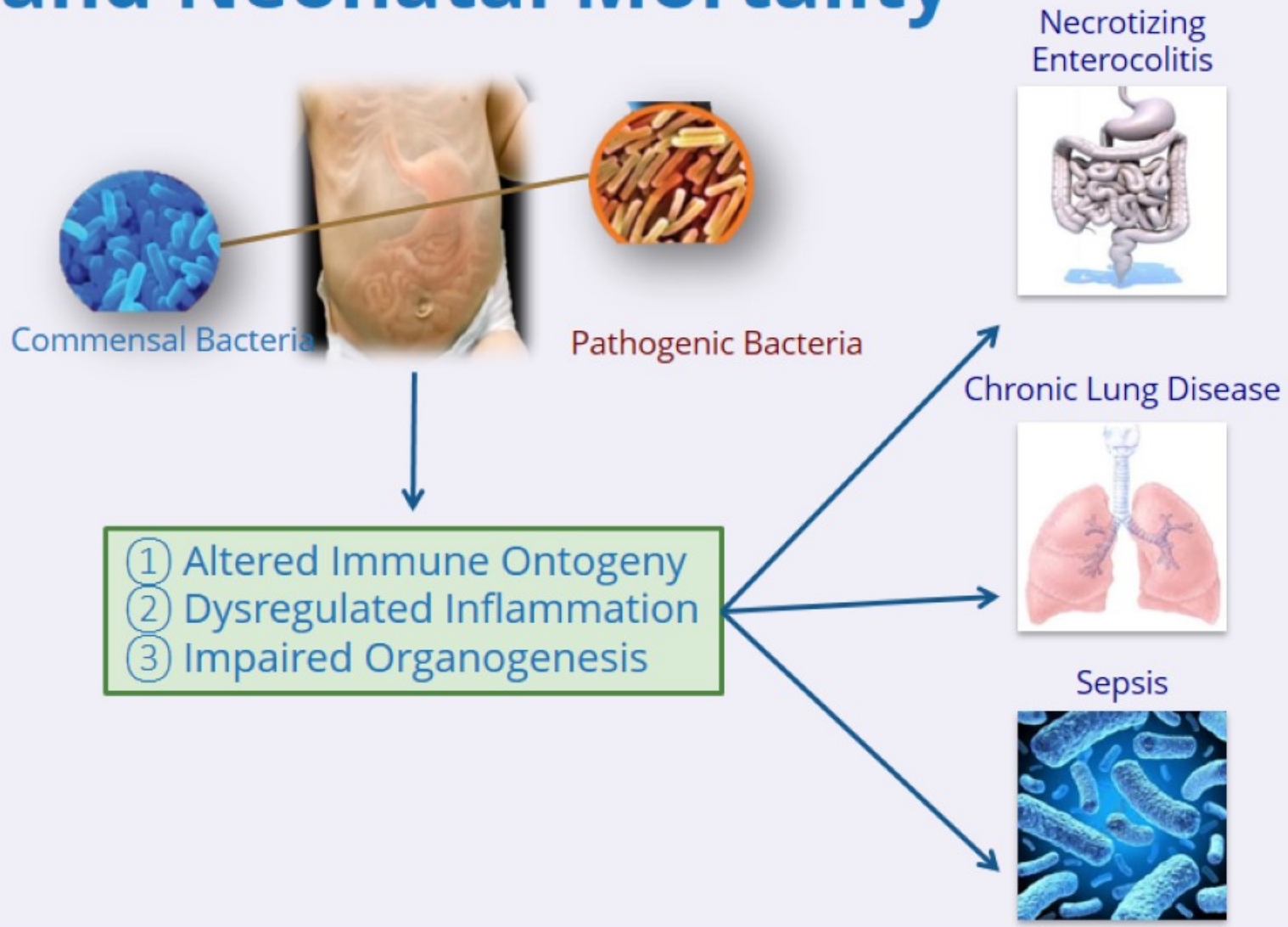
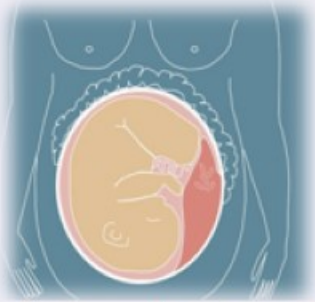


Image Credit: Sebastian Kaulitzki/Science Source

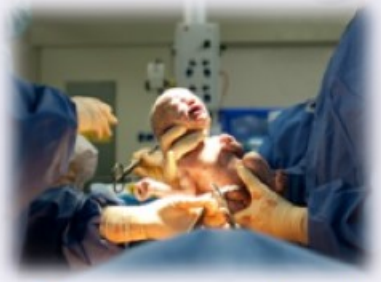
Dysbiosis and Neonatal Mortality



Influences on Postnatal Gut Development



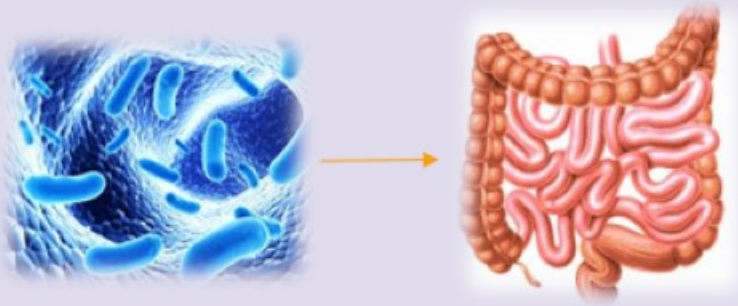
Maternal/Fetal Microbiome and Vaginal Delivery



Cesarean Section

Hospitalization

Skin-to-Skin



Medications

Breastfeeding

Delayed Feedings/Formula
Limited MM/DM

DM, donor milk; MM, mother's milk.

Breast Milk Increases Intestinal Barrier Function Compared to Formula

TABLE 2. COMPARISON OF MEDIAN L/M RATIOS (RANGE) BETWEEN INFANTS RECEIVING ANY HUMAN MILK AND THOSE RECEIVING ONLY FORMULA

	Median L/M ratio (range)			
Type of feeding	*Study time 1 (n = 47)*	*Study time 2 (n = 33)*	*Study time 3 (n = 20)*	*Composite*
Any human milk	0.076 (0.013–1.337)	0.167 (0.011–8.468)	0.178 (0.031–1.791)	0.343 (0.014–8.838)
Formula only	0.205 (0.062–2.178)	1.371 (0.218–30)	0.347 (0.247–1.887)	0.962 (0.576–32.525)

L/M, lactulose to mannitol.

Taylor SN, et al. *Breastfeed Med.* 2009;4:11-5. Used with permission from Mary Ann Liebert, Inc.

Influence of Diet on Intestinal Gene Expression

Breastfeeding

Table 2 Relative gene expression levels in breast-fed (BF) versus formula-fed (FF) infants following a 3-month feeding period

Gene	BF/FF	*P*-value	q-value
TACR1	1.80	0.0189	0.1670
REL	1.62	0.0047	0.1026
DUOX2	1.45	0.0215	0.1670
VAV2	1.36	0.0088	0.1404
NDST1	0.79	0.0103	0.1477
AOC3	0.78	0.0202	0.1670
SP2	0.76	0.0030	0.0860
IL1A	0.71	0.0089	0.1389
ALOX5	0.69	1.40E-05	0.0008
BPIL1	0.37	1.43E-05	0.0008
KLRF1	0.35	3.16E-05	0.0015

Fold change represents relative expression level in BF divided by FF infants for the 11 genes exhibiting the strongest multivariate relationships to microbiota virulence characteristics.

Formula:

- Lower phylogenetic heterogeneity (and decreased diversity) of the microbiome
- Lower overall gene expression by the intestinal epithelium

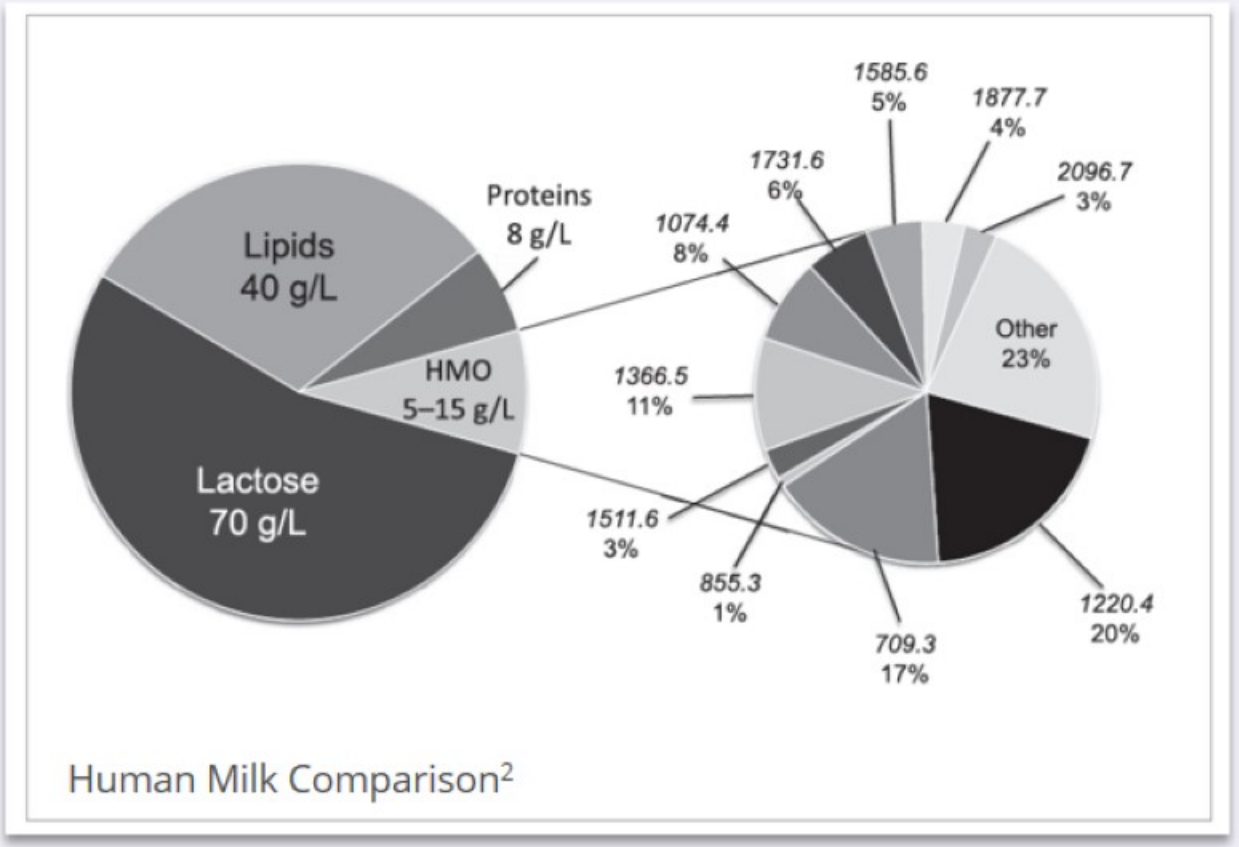
Gut motility, bacterial-mediated reactive oxygen species signaling, epithelial homeostasis

Mucosal inflammatory responses, permeability-increasing, vascular adhesion

RNA-seq on host RNA from shed intestinal cells in fecal samples.

Human Milk Oligosaccharides

- Diverse unconjugated glycans
- Abundant in and unique to human milk
- More than a hundred different HMOs have been identified
- Not every woman synthesizes the same set of oligosaccharides
- HMOs reach the distal small intestine and colon in an intact form and are excreted with the infant's feces[1]

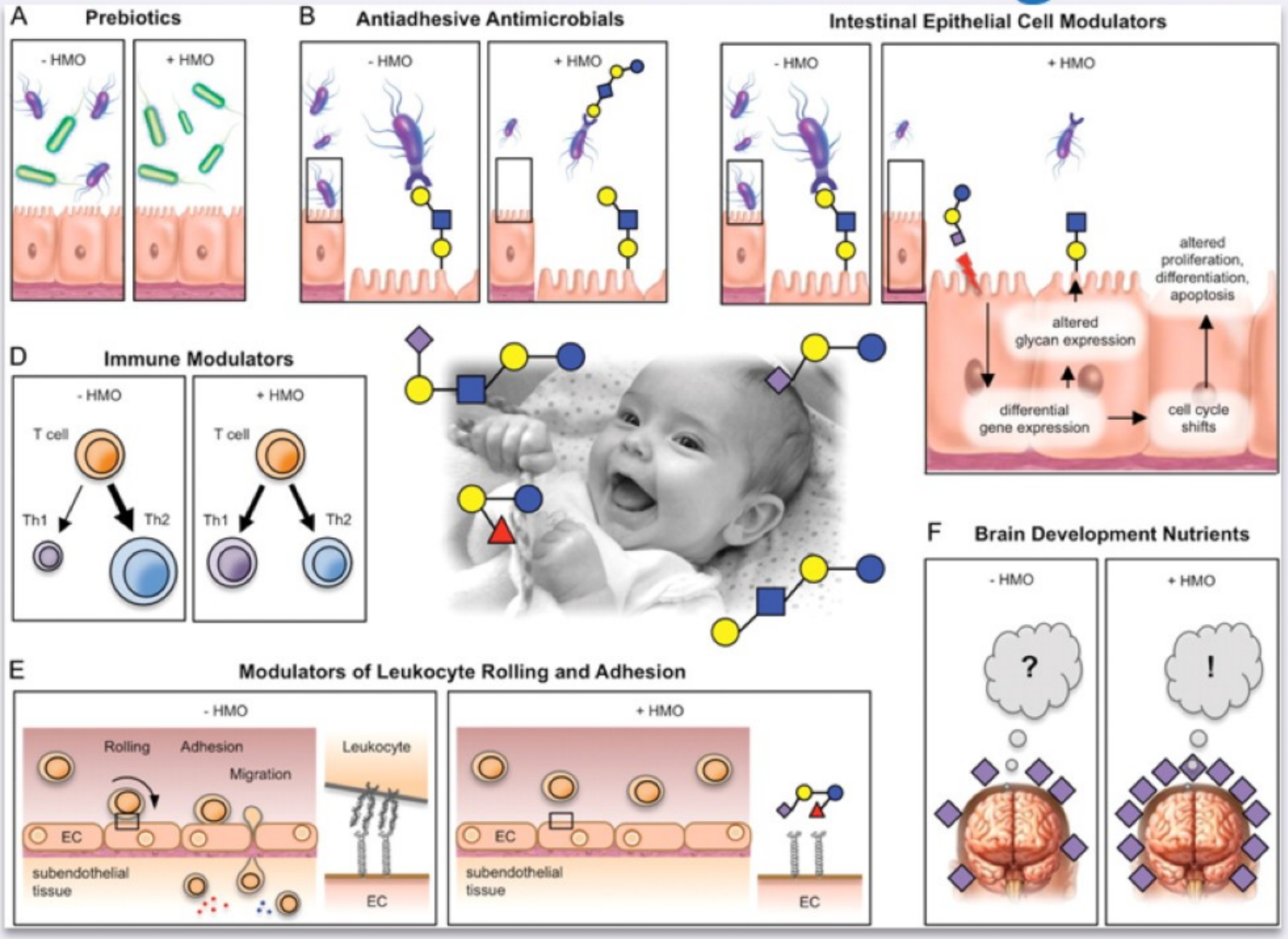


Human Milk Comparison[2]

HMOs, human milk oligosaccharides.

1. Bode L. *Glycobiology*. 2012;22:1147-62.
2. Zivkovic AM, et al. *Proc Natl Acad Sci USA*. 2011;108 Suppl 1:4653-8. © 2011 National Academy of Sciences. Used under terms for educational purpose.

Pleiotropic Effects of Human Milk Oligosaccharides



Bode L. *Glycobiology*. 2012;22:1147-62. Used with permission from Oxford University Press.

Delayed feedings/Formula
Limited MM/DM

Nutrition Practices

Table 5. Univariate Analysis of Fecal Cytokine Expression in Association with Early vs. Late Groups.

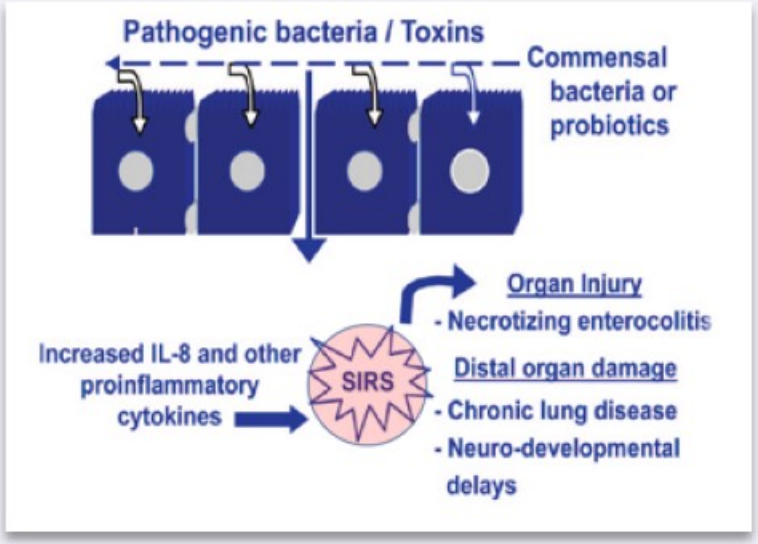
Variables	Early (p50±IQR)	Late (p50±IQR)
IL-8 (pg/ml)	1.9±3.2	6.1±22.8*
IL-1RA:IL-8	40.5±82.3	13.5±65.5*
IL-10:IL-8	8.2±1.2	5.9±1.6*

Table 6. Univariate Analysis of Serum Cytokine Expression (in pg/mL) in Association with Early vs. Late Group.

Variables	Early (p50±IQR)	Late (p50±IQR)
IL-1RA	175.8±316.2	316.2±569.1*
CRP	447.7±1227.4	948.8±4371.5*

Early = initial enteral feeding at the third postnatal day or less; Late = initial enteral feeding after the third postnatal day.

Evidence for Gut to Systemic Health Link



- Delayed feedings after 3 days leads to detectable inflammation at 2 weeks postnatal age and increased risk of CLD at 36 weeks PMA[1]
- Intestinal injury leads to sustained *systemic* inflammatory response; sustained systemic inflammatory response leads to poor neurocognitive outcomes, as does NEC[2,3]
- NEC a common node in clustering of neonatal morbidities[4]

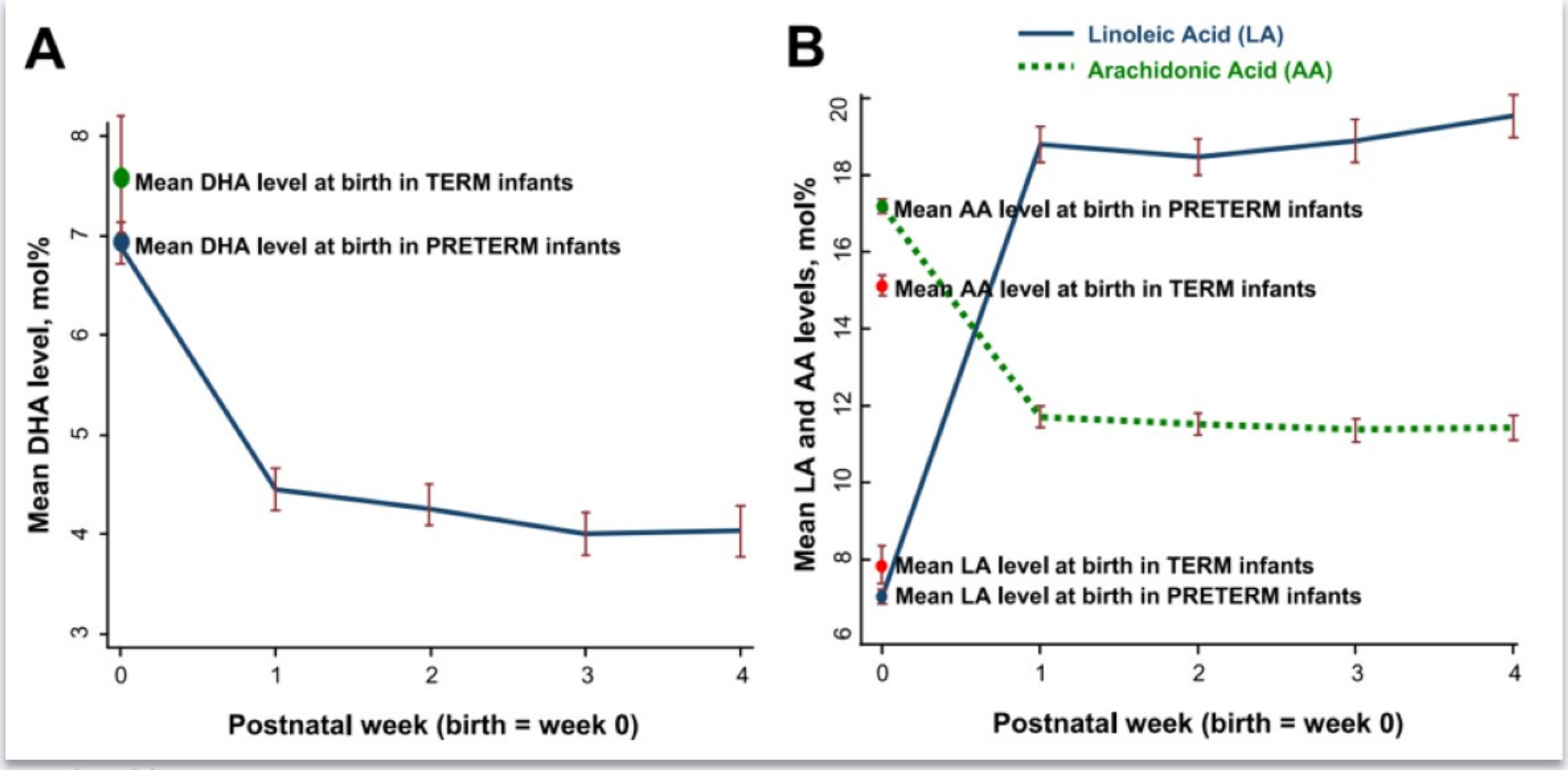
	Bowel	Brain	Retina	Lung
	NEC	VM/EL	ROP	BPD
Bowel		**2.3 (1.2, 4.3)**	**3.1 (1.7, 5.8)**	**3.7 (1.9, 7.1)**
Brain	14/6.9		1.1 (0.8, 1.6)	1.0 (0.6, 1.7)
Retina	30/14.5	61/50.8		2.6 (1.7, 3.9)
Lung	18/6	19/17	62/34	
Blood early	3/3.2	14/11.1	35/23.3	5/8.0
Blood late	17/12.6	52/44.1	118/92.6	42/31.6

- Humanized gnotobiotic mice with preterm microbiota results in dysregulated systemic inflammation and altered growth[5]

CLD, chronic lung disease; NEC, necrotizing enterocolitis; PMA, postmenstrual age; SIRS, systemic inflammatory response syndrome.

1. Konnikova Y, et al. *PLOS One.* 2015;10:e0132924. **2.** O'Shea TM, et al. *J Pediatr.* 2012;160(3):395-401.e4. **3.** Carlo WA, et al. *J Pediatr* 2011;159:919-25.e3. **4.** Leviton A, et al. *Acta Paediatr.* 2010;99:1795-800. **5.** Lu L, et al. *PLOS One.* 2015;10:e0124504.

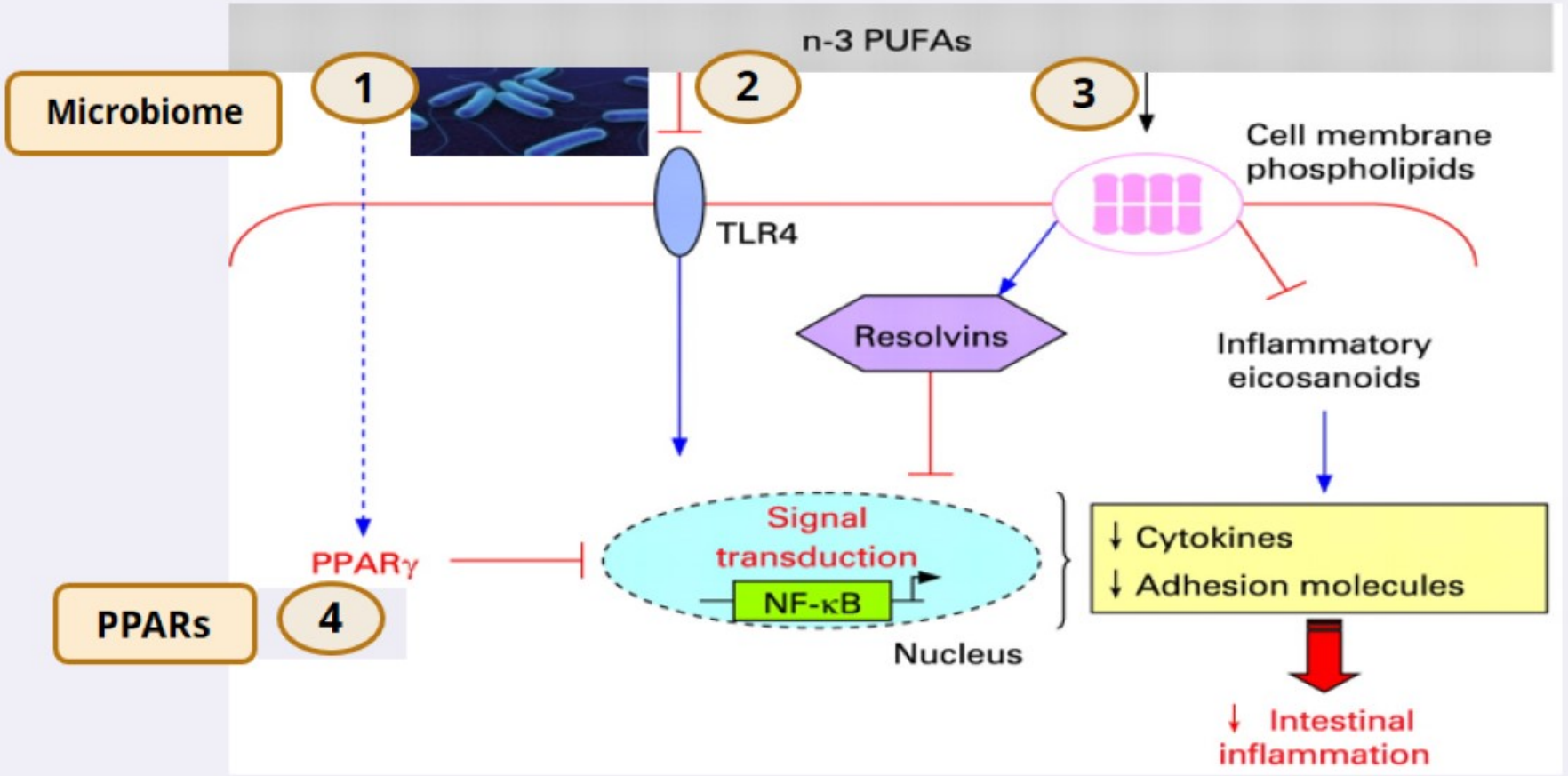
Nutrient-Specific Postnatal Transitions in the Preterm Infant Case Study: Long-Chain Polyunsaturated Fatty Acids



DHA, docosahexaenoic acid.

Martin CR, et al. *J Pediatr.* 2011;159:743-749.e1-2.

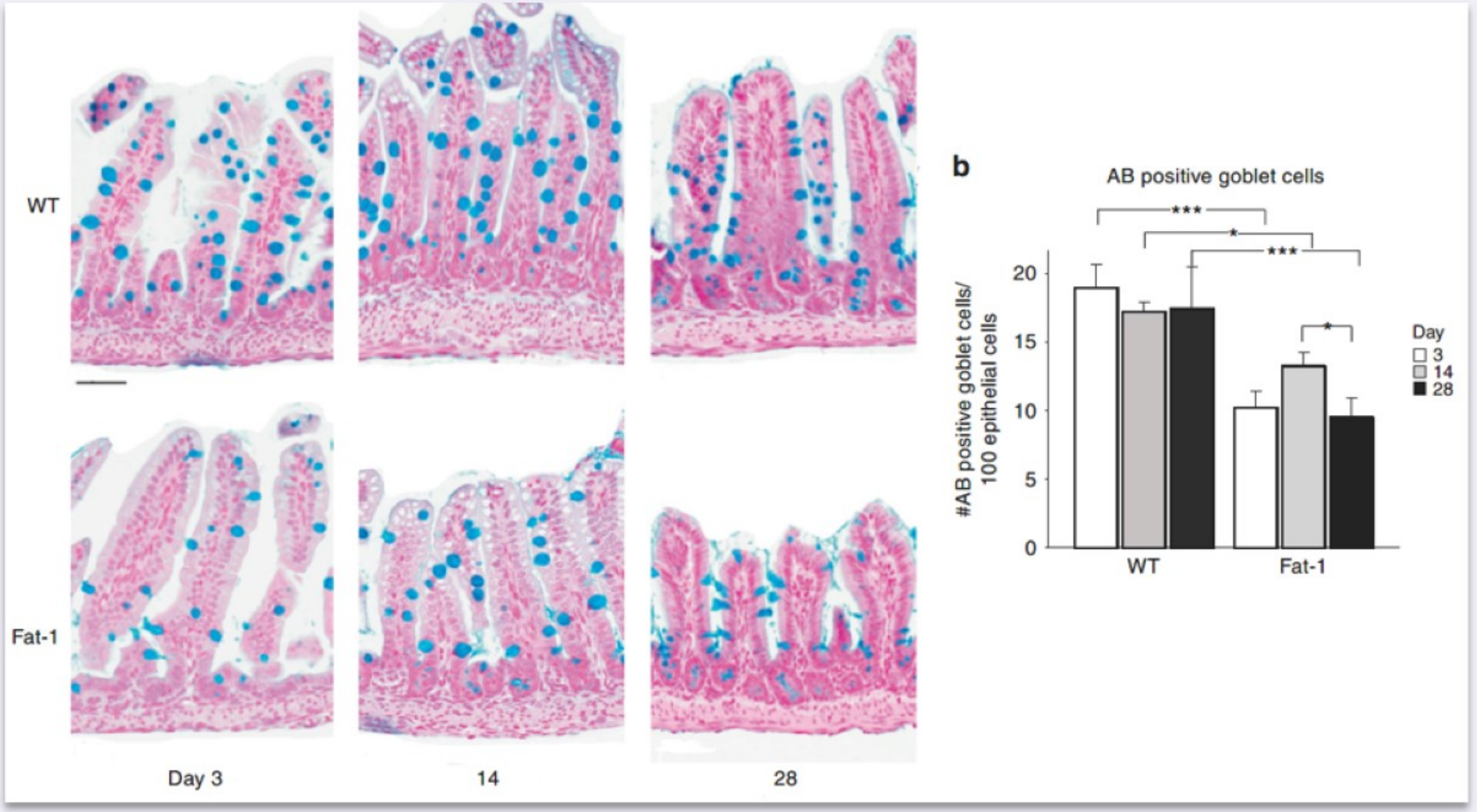
n-3 PUFAs and Gut Health



PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids.

Modified from Marion-Letellier R, et al. *Gut.* 2009;58:586-93.

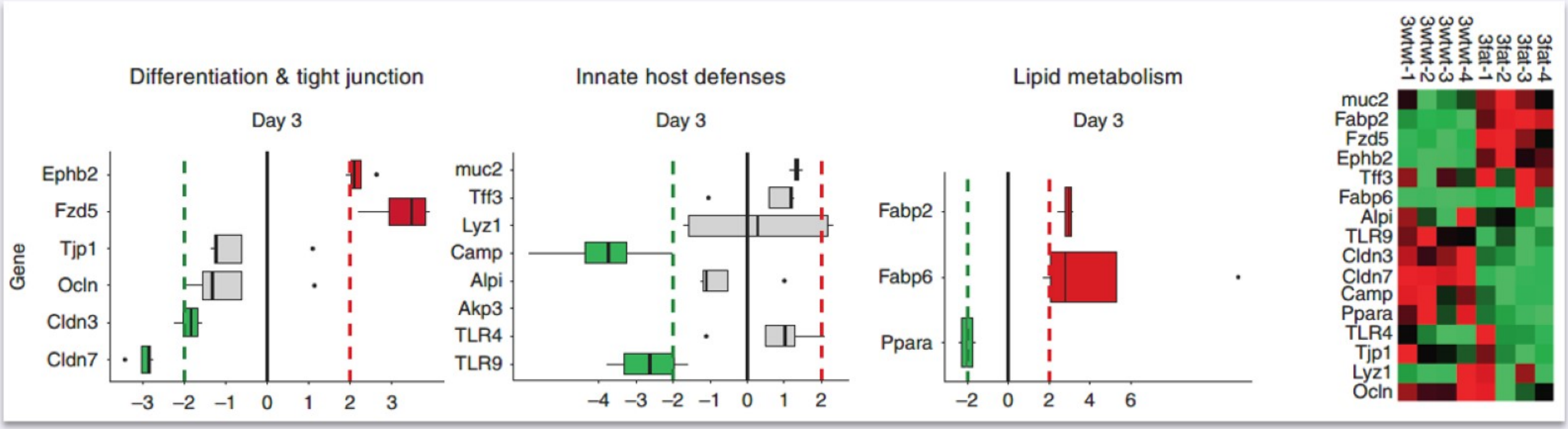
n-3 Dominant Fatty Acid Profiles Decrease Number of Goblet Cells



AB, Alcian Blue; Fat-1, fat-1 transgenic mice; WT, wild-type mice,

Singh P, et al. *Pediatr Res.* 2019;85:556-565. Used with permission from Springer Nature.

n-3 Dominant Fatty Acid Profiles Increase Cell Differentiation Markers, Decrease Genes Regulating Tight Junction



Singh P, et al. *Pediatr Res.* 2019;85:556-565. Used with permission from Springer Nature.

Nutrient Specific Impact on Gut Development

- As a package of care (breast milk vs other), or as specific elements, direct impact on the intestinal environment (microbiome/inflammation) and development that determine the balance between health and disease
- Not just local, for the gut, also systemic ramifications
- We should not presume safety, or likely no harm, with these bioactive molecules
- Need to understand dose, balance, windows of opportunity, and best delivery strategies

NUTRITION AND LUNG DEVELOPMENT

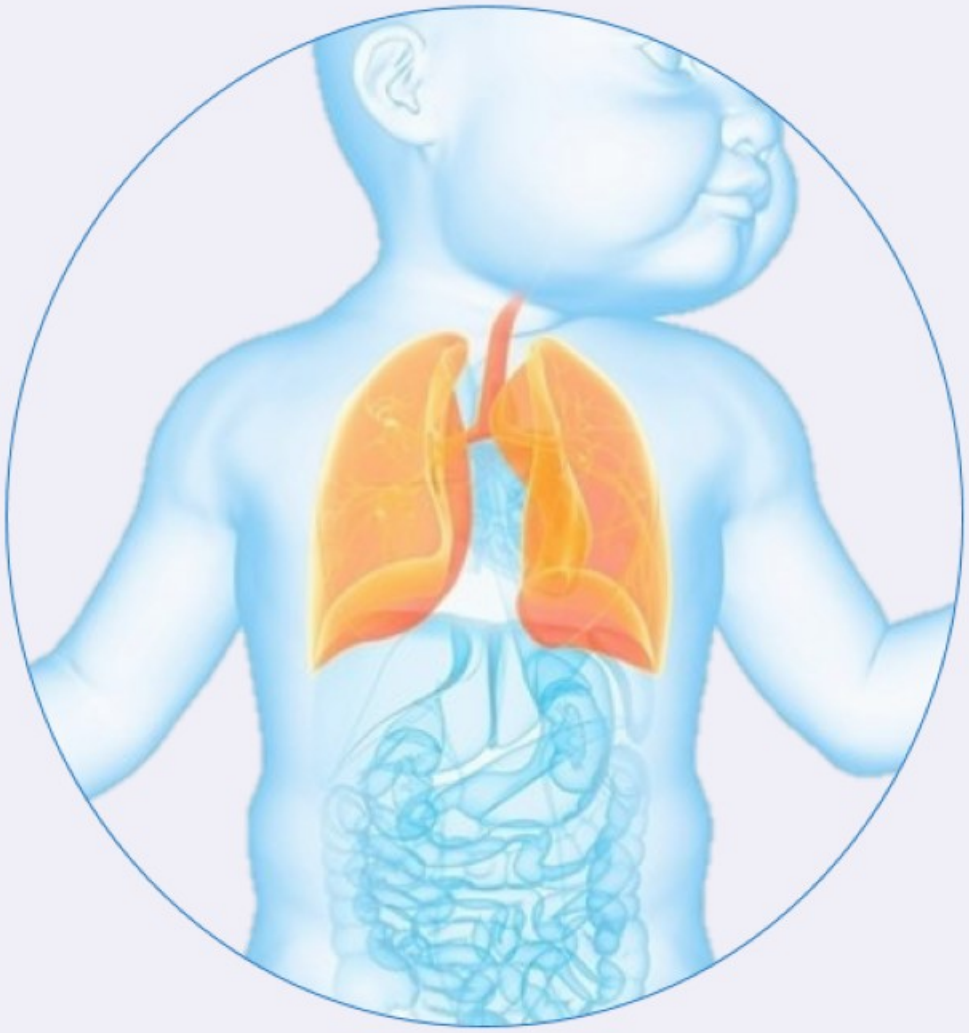


Image Credit: Sebastian Kaulitzki/Science Source

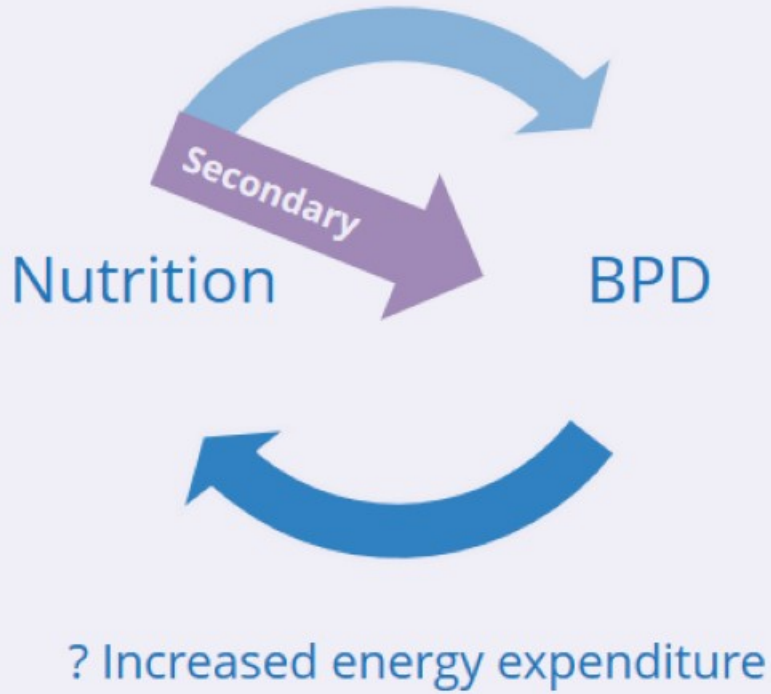
Epidemiologic Data Demonstrate that Growth Attainment in NICU is Associated with BPD Risk

TABLE 2 **Characteristics of Follow-up Cohort by Weight Gain Quartile**

Variable[a]	Quartile 1 ($n = 124$)	Quartile 2 ($n = 122$)	Quartile 3 ($n = 123$)	Quartile 4 ($n = 121$)	P[b]
Weight gain, mean (SD), g/kg per d	12.0 (2.1)	15.6 (0.8)	17.8 (0.8)	21.2 (2.0)	—
BPD, %	56	41	30	31	<.001

BPD, bronchopulmonary dysplasia.

Nutrition and BPD: Mechanisms?

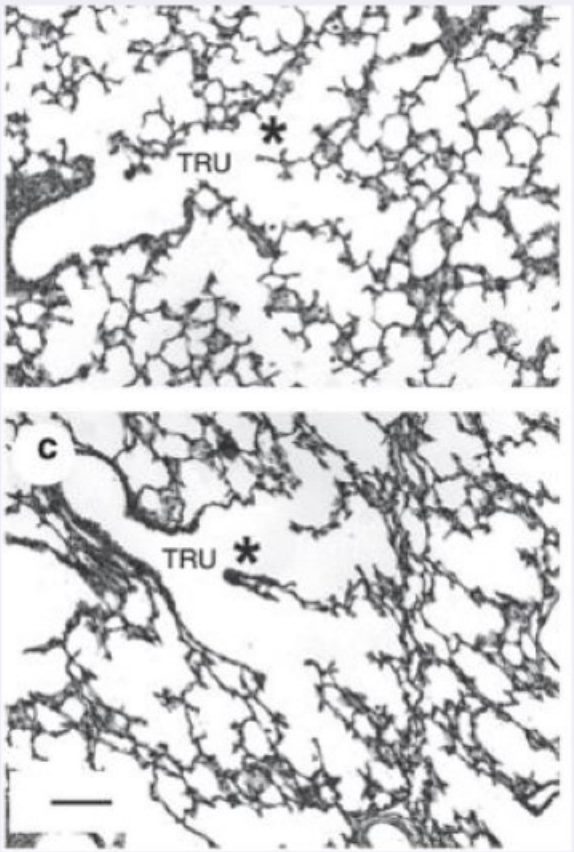


- **Is nutrition driving the lung disease** (A), or **is the lung disease driving the nutritional intake** (B)
- Role of nutrition:
  - **Primary**—Direct evidence that a specific nutritional parameter or nutrient is in the pathway of lung injury or repair
  - **Secondary**—A proxy to our practices around lung disease (fluid restriction, diuretics, steroids)

BPD, bronchopulmonary dysplasia.

Preclinical Animal Data

Restricted Nutrition/Postnatal Growth Restriction



Alveolar formation is dysregulated by restricted nutrition

- Lamb
- NIS vs NIS + RN; RN = lower fluids, fat, protein, calories (150 kcal/k/d v 60 kcal/k/d)
- 21 day model
- RN = reduced alveolar count, increased septal wall thickness, decreased caspase-3 (apoptosis), decreased PCNA (proliferation)
- Unable to determine specific nutrient effects; or windows of opportunity/vulnerability

NIS, noninvasive support; PCNA, proliferating cell nuclear antigen; RN, restricted nutrition.

Joss-Moore LA, et al. *Pediatr Res.* 2016;80:719-728. Used with permission from Springer Nature.

Base Diet—Breast Milk

Beyond Necrotizing Enterocolitis Prevention: Improving Outcomes With an Exclusive Human Milk–Based Diet[1]

- Multicenter, *retrospective cohort*
- Pre-, Post-exclusive HUM diet
- n= 1,587
- BPD defined as need for oxygen at 36 weeks PMA
- BOV 56.3% vs HUM 47.7% (p=0.0015)

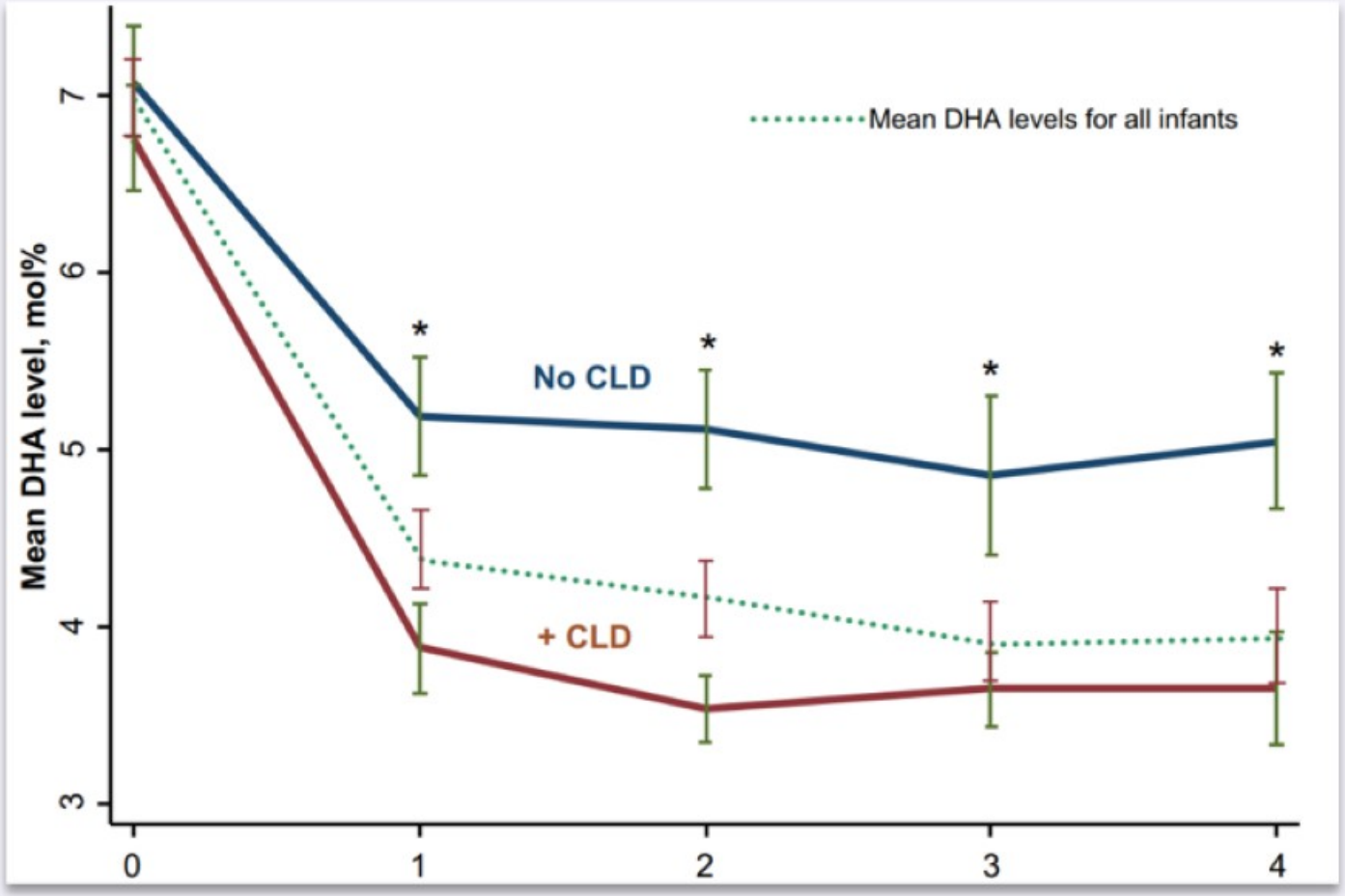
Does Breastmilk Influence the Development of BPD?[2]

- Multicenter, *prospective cohort*
- Exclusive formula vs exclusive breast milk
- n= 462
- BPD defined as need for oxygen at 36 weeks PMA + moderate/severe categories as defined by the NIH
- Formula 20.9% vs BM 11.2% (p=0.005)

BM, breast milk; BOV, bovine-based diet; BPD, bronchopulmonary dysplasia; HUM, human milk-based diet; PMA, postmenstrual age.

1. Hair AB, et al. *Breastfeed Med.* 2016;11:70-4. **2.** Spiegler J, et al. *J Pediatr.* 2016;169:76-80.e4.

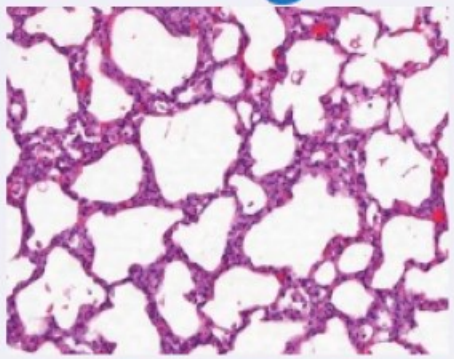
Low Blood Levels of DHA Associated With Increased Risk of BPD



BPD, bronchopulmonary dysplasia; CLD, chronic lung disease; DHA, docosahexaenoic acid.

Martin CR, et al. *J Pediatr*. 2011;159:743-749.e1-2.

DHA *and* AA Terminal Mediators Reduce Hyperoxia-Induced Changes in Lung Development

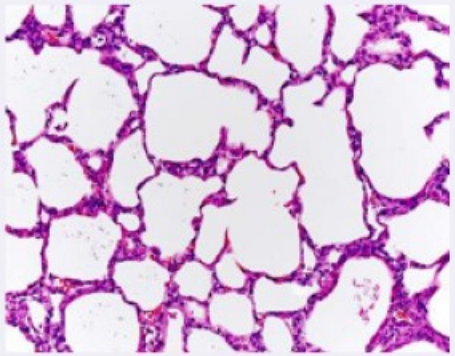


LXA4

RvD1

(-) Inflammation

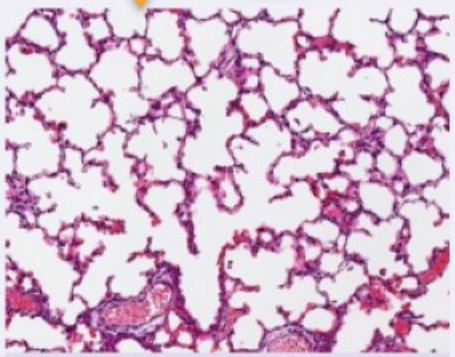
(-) TIMP1



Decreased SWT

(+) TGFb2

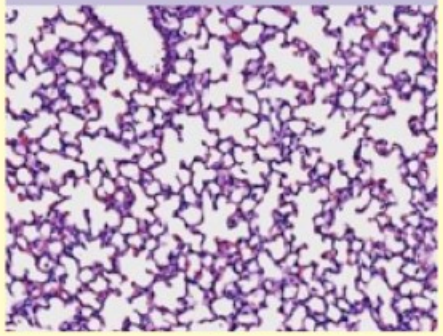
LXA4



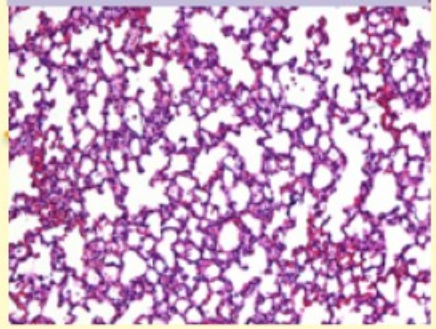
Increased Alveolarization

- Resolvin D1 (DHA product) reduces lung inflammation
- Lipoxin A4 (AA product) reduces lung inflammation and improves lung (alveoli) development
- Together, protect against lung injury in face of high oxygen exposure

Room Air



RvD1 & LXA4



Martin CR, et al. *PLOS One.* 2014;9:e98773.

Nutrition Interfaces Throughout Lung Development, Injury, and Repair

- T1 preclinical/animal evidence strongly links nutrition with lung development and disease pathogenesis
- Epidemiology studies and small clinical trials support nutrition modulating disease
- T3 translation challenging mostly due to:
  - Lack of well-designed studies
  - Need to ensure adequate numbers of infants at highest risk
  - Not understanding the what, why, how, and when dynamic; no biomarkers of nutritional efficacy
  - Animal models incomplete representative of the preterm biology and competing exposures—antenatal to postnatal; and models may need to be iteratively reassessed as the disease changes

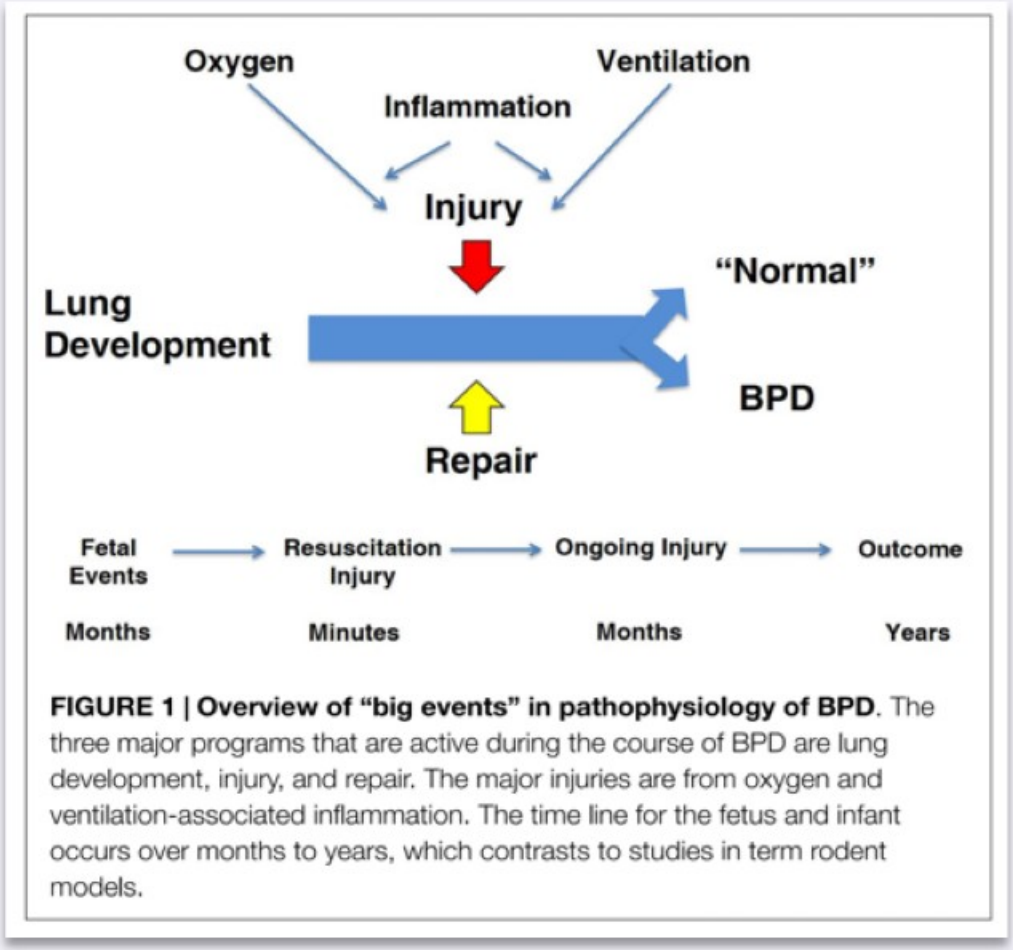


FIGURE 1 | Overview of "big events" in pathophysiology of BPD. The three major programs that are active during the course of BPD are lung development, injury, and repair. The major injuries are from oxygen and ventilation-associated inflammation. The time line for the fetus and infant occurs over months to years, which contrasts to studies in term rodent models.

Physiology and Development Driven by Lipid-Derived Nutrients

Lipids constitute an example of what needs to be considered when investigating nutrient driven research

- Must be thoroughly studied with the goal of rigor and reproducibility; just because it is a constituent of nutrition, breast milk or formula, cannot presume safety
- Giving in isolation or at a wrong dose, DHA—in and of itself or due to the ancillary effects to other fatty acids—may drive unwanted biological effects

DHA, docosahexaenoic acid.

Docosahexaenoic Acid and Bronchopulmonary Dysplasia in Preterm Infants

Table 2. Primary Outcome and Secondary Respiratory-Related Outcomes.*

Outcome	DHA Group (N=592)	Control Group (N=613)	Adjusted Effect (95% CI)	Adjusted P Value
Physiological BPD: primary outcome — no. (%)†	291 (49.1)	269 (43.9)	1.13 (1.02–1.25)	0.02
Physiological BPD or death before 36 wk of postmenstrual age — no./total no. (%)†‡	330/631 (52.3)	298/642 (46.4)	1.11 (1.00–1.23)	0.045
Clinical BPD — no./total no. (%)	315/592 (53.2)	304/612 (49.7)	1.09 (1.00–1.18)	0.06
Severity of BPD				
Mild — no. (%)†§	80 (13.5)	108 (17.6)	0.76 (0.58–0.99)	0.04
Moderate — no. (%)†§	65 (11.0)	50 (8.1)	1.35 (0.95–1.92)	0.10
Severe — no./total no. (%)¶	202/592 (34.1)	194/612 (31.7)	1.07 (0.93–1.22)	0.36
Surfactant use — no./total no. (%)‡‖	533/631 (84.5)	516/642 (80.4)	1.05 (1.00–1.10)	0.06
Days of respiratory support**	41.5±28.7	40.4±27.7	1.02 (0.94–1.10)	0.63
Postnatal glucocorticoids — no./total no. (%)	128/604 (21.2)	132/622 (21.2)	0.98 (0.80–1.19)	0.81
Days of caffeine use††	61.9±19.3	60.7±18.9	1.01 (0.99–1.04)	0.29
Days of diuretic use††	4.5±13.2	5.2±13.7	0.70 (0.46–1.07)	0.10

DHA, docosahexaenoic acid.

A Word of Caution

Intravenous lipid emulsions providing DHA at doses similar to those given in our trial are being used to provide nutritional support during the transition to full enteral feeding in preterm infants, although with limited testing in clinical trials.[34,35] Our results raise questions about the safety of this strategy and suggest the need for further study.

DHA, docosahexaenoic acid.

Essentiality of Arachidonic Acid

- Growth and neurodevelopment[1–3]
- Primary fatty acid in preterm brains until early term[4]
- Reduction associated with 40% increase in nosocomial sepsis[5]
- Provision of its distal metabolite (Lipoxin A4) improves alveologenesis in murine hyperoxia induced lung injury;[6] reduced AA decreases alveologenesis[7]
- Reduction associated with increased risk of retinopathy of prematurity[8]

AA, arachidonic acid.

1. Carlson SE, et al. Proc Natl Acad Sci U S A 1993;90:1073-7. **2.** Alshweki A, et al. *Nutr J.* 2015;14:101. **3.** Hadley KB, et al. *Nutrients.* 2016;8:216. **4.** Martinez M. *Brain Res.*1992;583:171-82. **5.** Martin CR, et al. *J Pediatr.* 2011;159:743-749.e1-2. **6.** Martin CR. *AAP Neoreviews.* 2015;16. **7.** Lavoie JC, et al. *JPEN J Parenter Enteral Nutr* 2018. **8.** Löfqvist CA, et al. *JAMA Ophthalmol.* 2018;136:271-277.

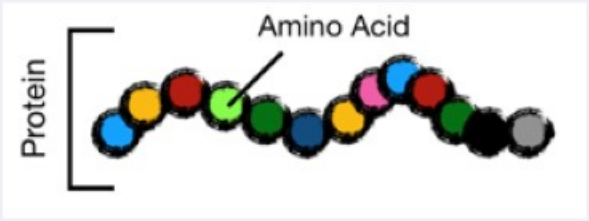
Understanding Nutrition at the Molecular Level

- Total calories (energy)
- Protein
- Fat

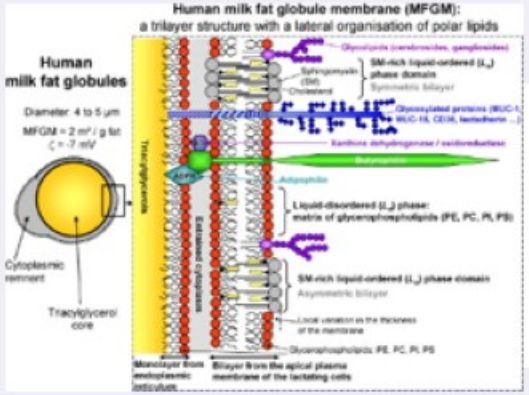
- Breast Milk

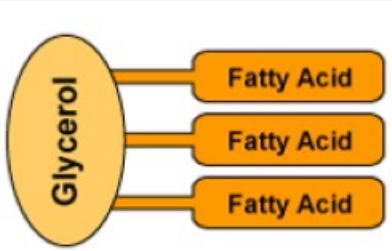
The rate of growth in NICU

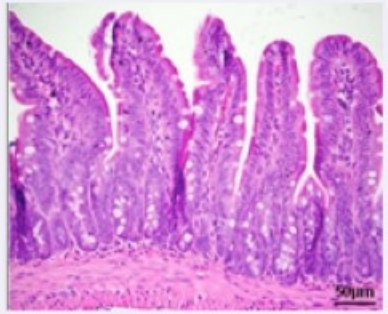
- Likelihood of disease *<u>while</u> in the NICU* (chronic lung disease)
- Neurocognitive development *<u>after</u> the NICU*

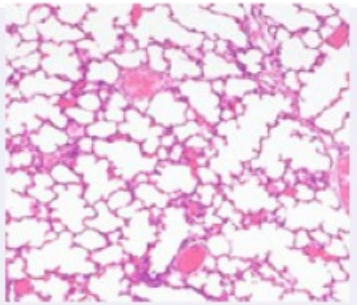


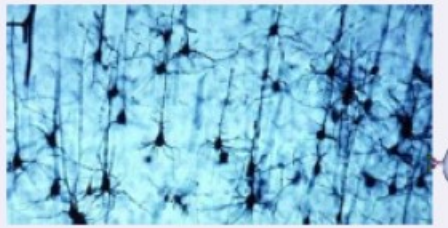
What? When? How?

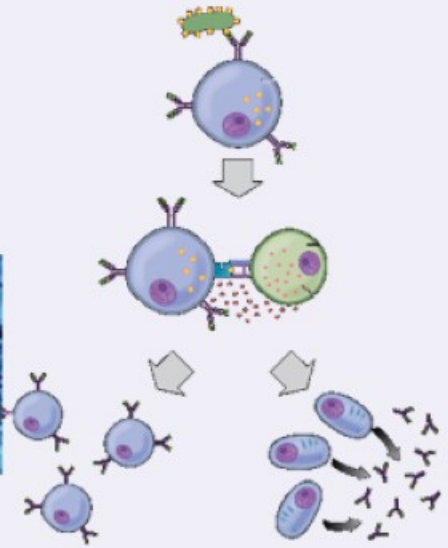












DEVELOPMENTAL ORIGINS AND THE BRAIN

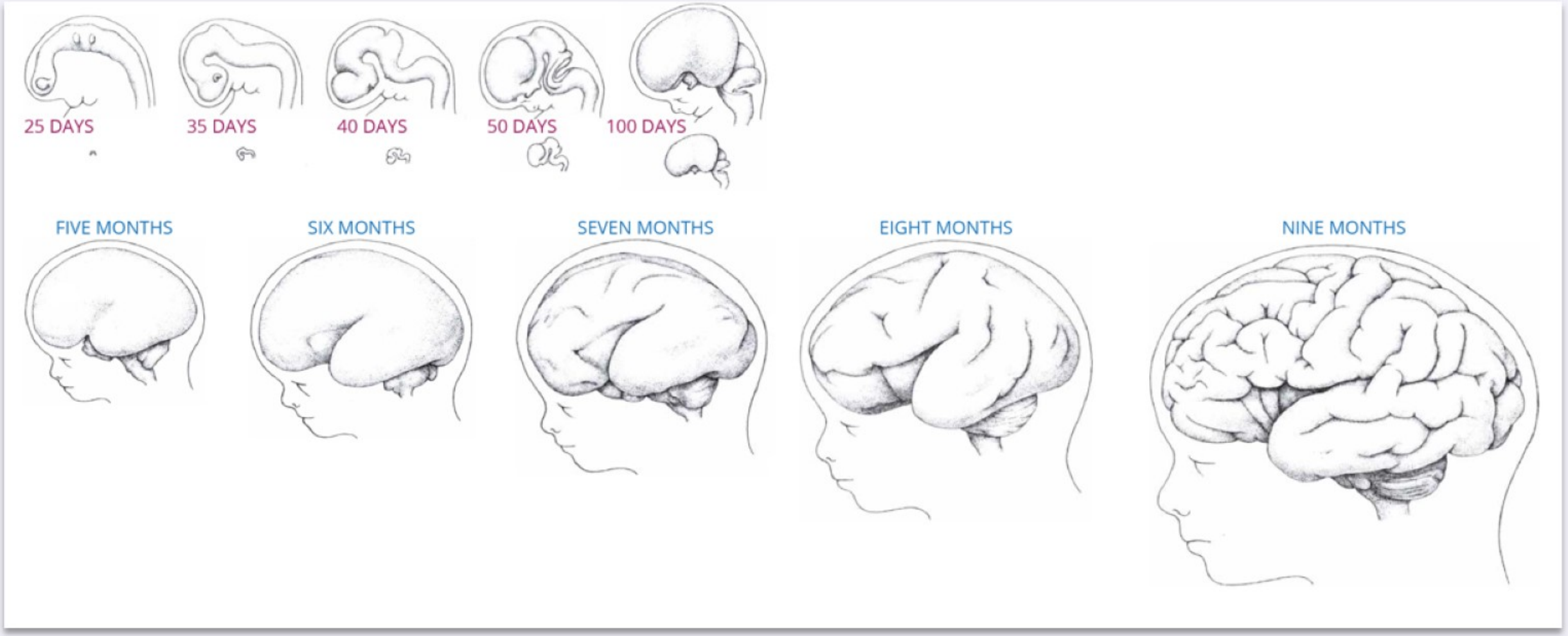
Early Neural Development Is Important—Immediately and Later

- **Early years** (fetal to 3 years): Development and sensitivity of early neural systems to extrinsic influences
  - **Primary systems**
    - Learning and memory (hippocampus/striatum)
    - Speed of processing (myelination)
    - Reward (dopamine/serotonin)
- Later developing higher order neural systems: Rely on early developing neural systems
  - **Prefrontal Cortex**
    - Initial connectivity from HC, striatum (early in life)
      - » **Examples:** Prematurity, IUGR, newborn ID
    - Maintenance (throughout development)
      - » **Example:** Preschool development programs

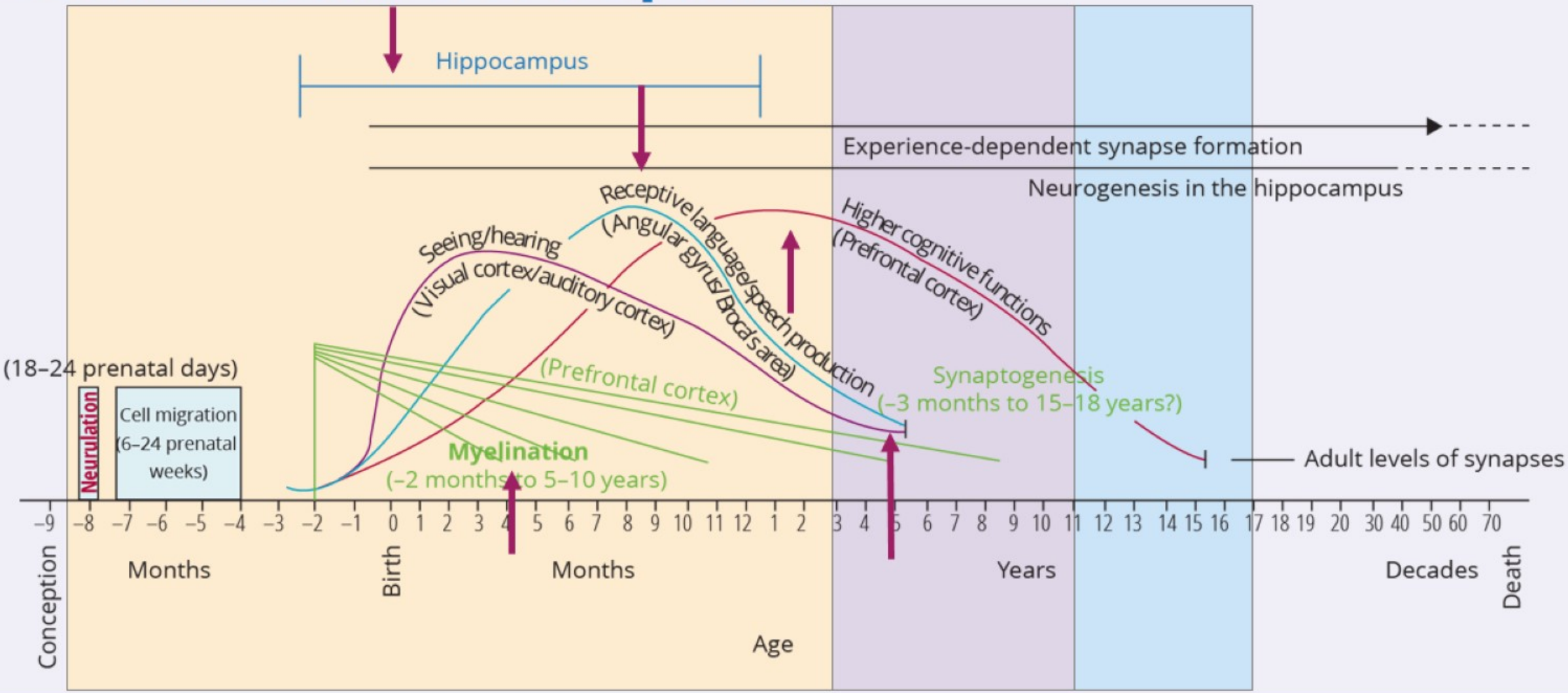
HC, hippocampus; ID, iron deficient; IUGR, intrauterine growth restriction.

Wachs TD, et al. *Ann N Y Acad Sci.* 2014;1308:89-106.

Developing Human Brain



Human Brain Development



Thompson RA, Nelson CA. *Am Psychol*. 2001;56:5–15. Reproduced with permission of the American Psychological Association.

Role of Nutrition in Brain Development

- Brain is developing in the late fetal and early neonatal period
  - Regionalized process
    - At risk: Hippocampus, myelination, neurotransmitters
- Highly metabolic process
  - 60% of total body O_2 consumption†
  - Reliant on metabolic substrates (nutrients) that support metabolism (eg, O_2, glucose, amino acids, iron, copper, iodine)

†Kuzawa CW. *Am J Phys Anthropol.* 1998;Suppl 27:177-209.

Nutrients and Brain: Importance of Timing

- Brain is not a homogenous organ
  - Regions (cortex, hippocampus, striatum, cerebellum)
  - Processes (myelin, neurotransmitters)
- All have different developmental trajectories
- Vulnerability to nutrient deficit is based on
  - When nutrient deficit occurs
  - Region's requirement for that nutrient at that time

Nutrients That Affect Early Brain Development and Later Adult Function

Macronutrients

- Protein[1,2]
- Fats (LC-PUFA)[1,2,3]
- Glucose[1,2]

Micronutrients

- Iron[1,2,3]
- Zinc[1,2]
- Copper[1,2]
- Iodine (Thyroid)[1,2]

Vitamins/Cofactors

- B vitamins (B6, B12[1])
- Vitamin A
- Vitamin K
- Folate[1,2,3]
- Choline[1,2,3]

[1]Exhibits critical/sensitive period for neurodevelopment
[2]Early deficiency results in long-term dysfunction
[3]Evidence for epigenetic mechanism

Cusick SE, et al. *J Pediatr.* 2016;175:16–21.

Examples of Nutrients and Regional vs Global Perinatal Brain Effects

Nutrient	Brain Requirement for Nutrient	Affected Areas
Protein-energy	Cell proliferation, Cell differentiation, Synaptogenesis, Growth factors	Global Cortex Hippocampus
Iron	Myelin Dopamine Energy	White matter Striatal-frontal Hippocampal-frontal
Zinc	DNA Neurotransmitter release	Autonomic NS Hippocampus Cerebellum
LC-PUFAs	Synaptogenesis Myelin	Eye Cortex

ANS, autonomic nervous system; LC-PUFAs, long-chain polyunsaturated fatty acids.

Georgieff MK. *Am J Clin Nutr.* 2007;85:614S-620S.

Evidence for Long-Lasting Effects of Early Nutritional Status on Brain in Humans

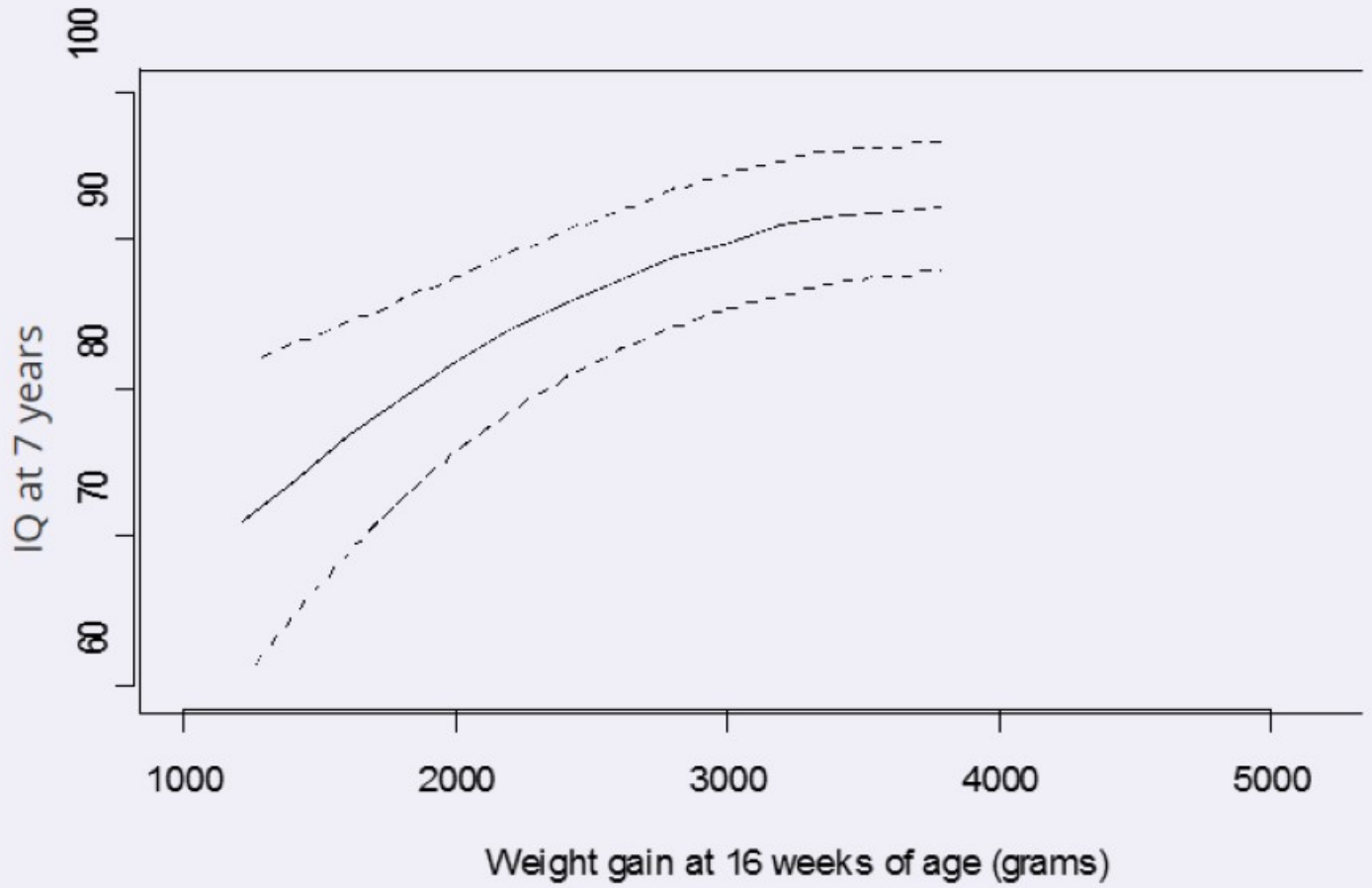
- Outcomes of IUGRs[1]
  - Lower IQ
  - Poorer verbal ability
  - Worse visual recognition memory
  - 15% with mild neurodevelopmental abnormalities
  - 30% increased risk of schizophrenia[2]

- **Guatemalan studies** show effects 25 years after protein supplementation in childhood[3]
- **Fetal iron deficiency** increases risk of
  - Schizophrenia[4]
  - Autism[5]
  - Depression/anxiety[6]
  - Poorer executive function[7]

ID, iron deficient; IUGR, intrauterine growth restriction.

1. Strauss RS, et al. *J Pediatr.* 1998;133(:67-72. **2.** Eide MG, et al. *Psychol Med.* 2013;43:2057-66. **3.** Pollitt E, et al. *J Nutr.* 1995;125:1111S-1118S. **4.** Insel BJ, et al. *Arch Gen Psychiatry.* 2008;65:1136-44. **5.** Schmidt RJ, et al. *Am J Epidemiol.* 2014;180:890-900. **6.** Lozoff B, et al. *Pediatrics.* 2000;105:E51. **7.** Lukowski AF, et al. *Nutr Neurosci.* 2010;13:54-70.

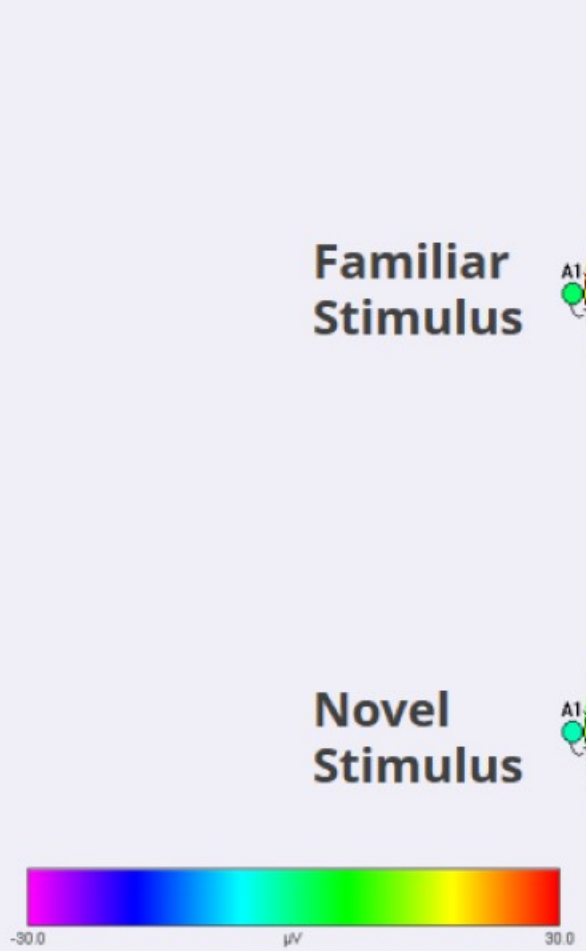
Effect of Postnatal Failure to Gain Weight after IUGR on 7-year IQ



IUGR, intrauterine growth restriction.

Pylipow M, et al. *J Pediatr.* 2009;154:201-6.

Long-Term Effects of Newborn ID at 3.5 Years



ID, iron deficient.

Infants who were iron deficient as newborns have a differently wired brain and process memory events differently **even after iron repletion**

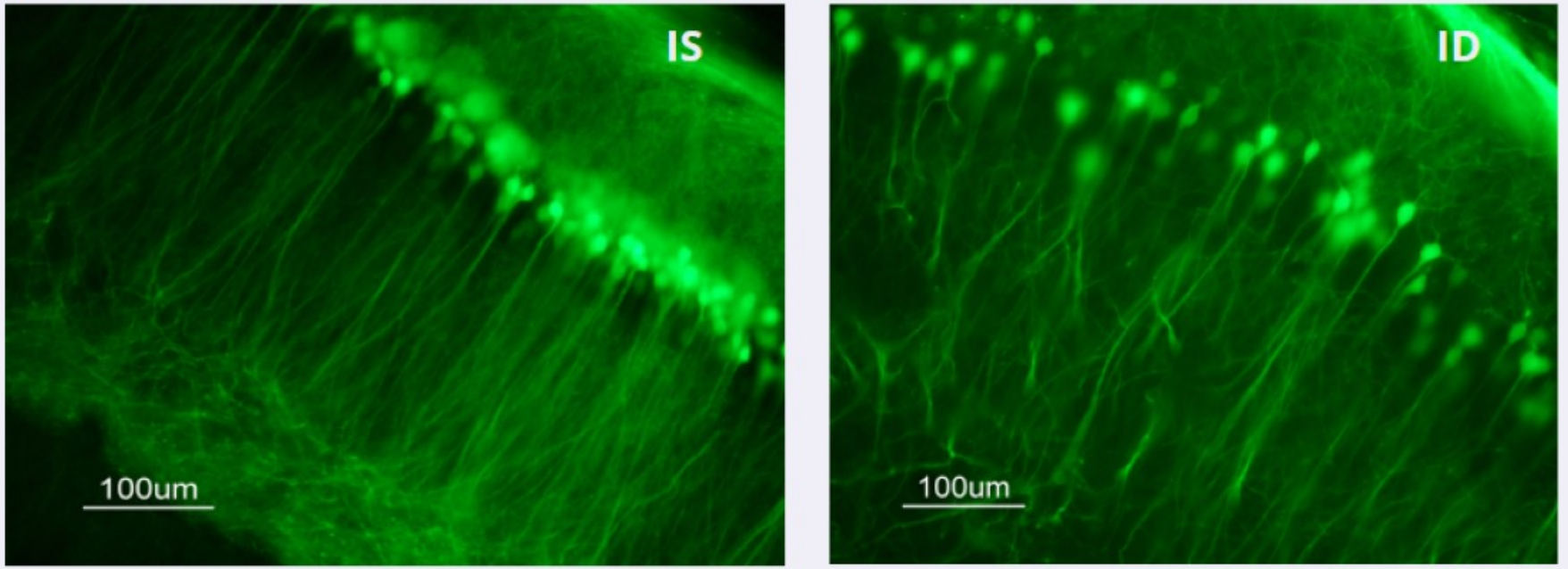
Fetal ID disrupts neonatal learning and memory

Heat maps courtesy Michael K. Georgieff, MD, Center for Neurobehavioral Development. Photo Credit: Jon Wilson/Science Source

Two Major Theories for Long-term Loss of Synaptic Plasticity

1 Residual Structural Deficits

- Nutrient deficiencies during critical periods of development result in permanent structural change[1-4]
- Disordered neuronal structures relate to neurobehavioral defects[5,6]



IS, iron sufficient; ID, iron deficient.

1. Hensch TK. *Annu Rev Neurosci.* 2004;27:549-79. **2.** Carlson ES, et al. *J Nutr.* 2009;139:672-9. **3.** Fretham SJ, et al. *Hippocampus.* 2012;22:1691-702. **4.** Callahan LS, et al. *Dev Neurosci.* 2013;35:427-36. **5.** Jorgenson LA, et al. *Hippocampus.* 2005;15:1094-102. **6.** Pisansky MT, et al. *Hippocampus.* 2013;23:952-62.

Critical Periods

- As the brain ages, it loses plasticity and ability to recover
- Developing brain is highly vulnerable but also has greater plasticity
- Cellular basis of critical periods being elucidated in
  - Visual system, cortex, hippocampus, language nuclei (bird)

- Lower efficiency
- Higher plasticity
- More amenable to treatment

Critical period of rapid development

- Higher efficiency
- Lower plasticity
- Less amenable to treatment

Two Major Theories for Long-Term Loss of Synaptic Plasticity *(continued)*

2 Altered Regulation of Synaptic Plasticity Genes Through Epigenetic Modification

- Gene networks responsible for neurobehavioral performance and risk of psychopathology
- Specific genes: eg, BDNF
  - Critical for
    - Neuronal differentiation during development
    - Maintenance of adult plasticity
  - Epigenetically modifiable by
    - Fetal and neonatal stress
    - Early life nutrition

BDNF, brain-derived neurotrophic factor.

Martinowich K, et al. *Science.* 2003;302:890-3. Tyagi E, et al. *Neurobiol Dis.* 2015;73:307-18.

Epigenetic Modifications of Chromatin

- Methylation of CpG Islands
  - Methyl (CH_3) groups attach to "islands" of DNA where C (Cytosine) and G (Guanine) nucleotides are next to each other
    - More methylation; less DNA transcription → less protein
- Histone Acetylation and Methylation
  - Histones found outside of DNA nucleotides and wind around them
  - Histone status can "open up" gene to more transcription or "close it off" leading to less transcription
- Overall effects depend on whether genes are active or repressive
  - Difficult to make predictions on effect without mapping pathways

Nutrients, Epigenetics, and the Developing Brain

Several fetal/neonatal nutritional conditions associated with brain epigenetic modifications in rodents

IUGR	• Generalized fetal malnutrition: Responsible nutrients have not been isolated • Activation of glucocorticoids: Stress alters BDNF DNA methylation
LC-PUFA	• DNA methylation of BDNF
Methyl donors and DNA methylation	• Choline • Folate
Iron	• Iron deficiency, anemia (hypoxia), or both
Vitamin A	• Vitamin A supplementation reduces DNA methylation
Riboflavin	• Cofactor for small family of lysine histone demethylases
No current evidence for zinc, copper, iodine, selenium, B12, thiamine, other B vitamins, vitamin E, vitamin D	

BDNF, brain derived neurotrophic factor; IUGR, intrauterine growth restriction ; LC-PUFA, long-chain polyunsaturated fatty acids.

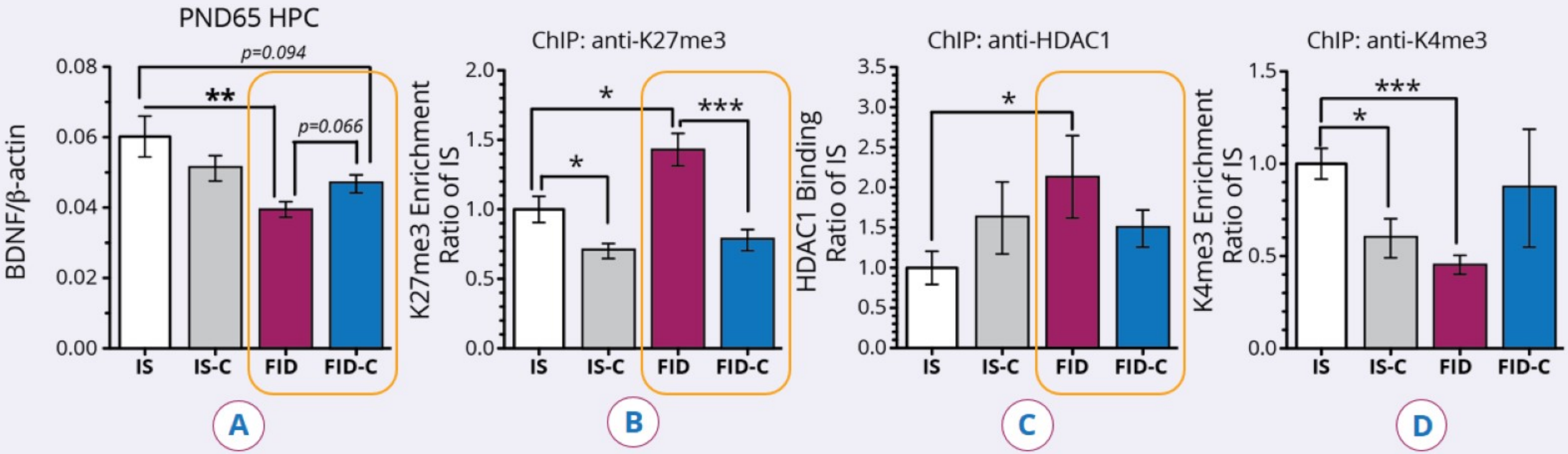
Ke X, et al. *Int J Dev Neurosci.* 2014;38:59-67. Grissom NM, et al. *Int J Dev Neurosci.* 2013;31:406–14. Ke X, et al. *Physiol Genomics.* 2010;42:177-89. Zeisel S. *Nutrients.* 2017;9.pii: E445; Ly A, et al. *J Nutr Biochem.* 2016;33:103–10; Cho CE, et al. *Epigenetics.* 2013;8:710-9; Barua S, et al. *Epigenetics Chromatin.* 2014;7:3; Langie SA, et al. *FASEB J.* 2013;27:3323-34. Blegen MB, et al. *Physiol Rep.* 2013;1:e00096; Tran PV, et al. *Am J Physiol Regul Integr Comp Physiol.* 2015;308:R276-82.

Prenatal Choline Supplementation

- Many populations do not get adequate iron in their diet
  - Grain based
  - Iron inhibitors (eg, phytates, tea)
- **Choline**
  - Common B-complex vitamin found in eggs, cruciferous vegetables
- Improved electrophysiology, biochemistry, brain morphology, learning, and memory when given during gestation
- Potential biological mechanisms
  - Acetylcholine (neurotransmitter)
  - Phosphotidylcholine (myelin component)
  - **Epigenetic modification (CH_3 donor)†**

†Zeisel S. *Nutrients.* 2017;9.pii: E445.

Prenatal Choline Rx Reverses Long-term Epigenetic Modifications Caused by Early Life ID



BDNF, brain-derived neurotrophic factor; ChIP, chromatin immunoprecipitation assay; FID, formerly iron deficient; HDAC, histone deacetylases; IS, iron sufficient.

Tran PV, et al. *Am J Physiol Regul Integr Comp Physiol.* 2015;308:R276-R282.

Refinement of the Barker Hypothesis

- Barker's associations stronger if difference between degree of IUGR and rapidity of postnatal growth is considered[1]
  - High weight gain in first year after IUGR
  - Isn't that "catch-up growth"?
- Concept of a "thrifty phenotype" *in utero* = "fetal anticipation"[2]
  - Designed to preserve vital systems during periods of relative nutrient insufficiency (IUGR)
    - Caloric demand of human fetal brain, up to 60%[3]
    - Programming of peripheral muscle insulin resistance to divert glucose to brain
  - Not designed to handle sudden large amounts of nutrient delivery (rapid postnatal re-feeding)

IUGR , intrauterine growth restriction.

1. Singhal A, et al. *Lancet.* 2004;363:1642-5. **2.** Gluckman PD, et al. *Science.* 2004;305:1733-6. **3.** Kuzawa CW. *Am J Phys Anthropol.* 1998;Suppl 27:177-209.

Fetal Programming in IUGR: Is Catch-up Growth a Good Idea?

- Term, SGA infants given a "growth promoting formula" had higher diastolic BP at 6–8 years of age compared to those on standard formula†
- Rat model of maternal protein restriction during pregnancy:
  - Feeding extra protein for catch-up growth resulted in early adult death, especially in males

SGA, small for gestational age.

†Singhal A, et al. *Lancet.* 2004;363:1642-5.

Does the Postnatal Growth Pattern After IUGR Affect Later IQ?

- Slow postnatal growth after IUGR decreases IQ even further[1,2]

- Does excessive growth also affect IQ?
  - 464 IUGR infants (<2.2 kg at >37 weeks)[2]
  - IQ as a function of postnatal weight gain in the first 4 postnatal months

IUGR, intrauterine growth restriction.

1. Casey PH, et al. *Pediatrics.* 2006;118:1078-86. **2.** Pylipow M, et al. *J Pediatr.* 2009;154:201-6.

Effect of Postnatal Excess Weight Gain After IUGR on 7-year IQ

IUGR, intrauterine growth restriction.

Pylipow M, et al. *J Pediatr.* 2009;154:201-6.

Effect of Maternal Obesity on Offspring Mental Health

Excessive GWG

Maternal Obesity, High-Fat Diet, Metabolic Disease

Intrauterine Development

Early Post-natal Development

Inflammation
Sex Hormones
Metabolic Hormones
Excess Nutrients

Impaired Brain Development

↑Risk of Mental Health and Neurodevelopmental Disorders

Reproduced from Rivera HM, Christiansen KJ, Sullivan EL. *Front Neurosci*. 2015;9:194.

Clinical Implications

Preconception	
Weight management in women of child-bearing age	• Reduction of obesity
Nutrient sufficiency	• Not just macronutrients, but micronutrients • 25%–40% of women of CBA are iron deficient • Not just a low- and middle-income country problem

CBA, child-bearing age.

Clinical Implications *(continued)*

Gestation	

Blood pressure control	• 10% of population suffered IUGR
	• 75% of IUGR in US is due to maternal hypertension or preeclampsia during pregnancy
	  • 50% are iron deficient at birth
	  • All have protein malnutrition

Blood sugar control	• 10% of pregnancies complicated by maternal diabetes (pregestational or gestational) • 65% of infants of diabetic mothers are iron deficient at birth
Stress reduction	• Maternal stress → fetal stress → abnormal fetal brain development (and iron deficiency)
Weight management	• Reduction of obesity
Nutrient sufficiency	• Prenatal vitamins, including iron • LC-PUFA (DHA) supplementation

IUGR, intrauterine growth restriction; LC-PUFA, long-chain polyunsaturated fatty acids; DHA, docosahexaenoic acid.

Clinical Implications *(continued)*

Postnatal (especially 0–3 years)	
Nutrition	• **BREAST MILK** • LC-PUFA (DHA) supplementation of formula-fed babies • Maintain iron and zinc sufficiency • Screen for thyroid status
Avoidance/reduction of glucocorticoid steroid use	• Steroids alter brain development • Steroids alter how critical nutrients are accreted
Reduce infectious burden	• Infection and inflammation alter brain development during critical periods of growth

LC-PUFA, long-chain polyunsaturated fatty acids; DHA, docosahexaenoic acid.