Gut Microbiota: PNEI Director of Developmental Age

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Received 27 July, revised 25 August, accepted 27 August
Online first: 30 September 2026

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Please cite: Lombardi Mistura E. (2026). Gut Microbiota: PNEI Director of Developmental Age. *PNEI Review.*
DOI: 10.3280/pnei2026oa23591

Abstract. *The epistemological transition of modern pediatrics is marked by the overcoming of the reductionist model in favor of a systemic and integrated vision of the human organism. In this scenario, Psychoneuroendocrinoimmunology (PNEI) offers the theoretical framework to understand how adult health is programmed during the very early stages of ontogenetic development (Bottaccioli, Bottaccioli, 2020), and the gut microbiota no longer represents a cluster of commensal microorganisms but a true dynamic, plastic, and systemic psychoneuroendocrinoimmunological organ, capable of acting as the undisputed director of the pathway (Lozupone* et al.*, 2012). Through the use of omics sciences (metagenomics, transcriptomics, metabolomics), contemporary research has revealed the functional profile of this microbial ecosystem (Turnbaugh* et al.*, 2009; Milani* et al.*, 2017), demonstrating how its chemical metabolites integrate into the host's regulatory networks. This article delves into the biochemical and molecular mechanisms through which the microbiota modulates the bidirectional communication axes between the gut, immune system, endocrine system, and central nervous system in the first 1000 days of life (Cryan* et al.*, 2019).*

Keywords: *gut microbiota, psychoneuroendocrinoimmunology (PNEI), developmental age, first 1000 days, early dysbiosis, epigenetics.*

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Introduction and Paradigm Shift: PNEI Network and Pediatric Evolution

Contemporary medicine is experiencing a profound conceptual revolution, marked by the progressive decline of the Cartesian biomedical approach, which for centuries compartmentalized the human body into isolated organs and systems. This old reductionist paradigm has proven insufficient to explain the epidemiological explosion of non-communicable chronic diseases (NCDs), neurodevelopmental disorders, allergies, and metabolic dysfunctions that affect the pediatric population at an increasingly early age. Health and disease must be re-read through the lens of systemic complexity (Bottaccioli, Bottaccioli, 2020).

Psychoneuroendocrinoimmunology (PNEI) stands at the center of this paradigm shift as a scientific discipline that studies the bidirectional relationships between the psyche (cognitive and emotional processes) and the major biological regulation systems: the nervous system, the endocrine system, and the immune system (Bottaccioli, Bottaccioli, 2020).

In this integrated scenario, the discovery of the gut microbiota and its characterization as a "PNEI organ" are of extraordinary interest. Indeed, the gut microbiota actively participates in the modulation of the hypothalamic-pituitary-adrenal (HPA) axis (Sudo *et al.*, 2004), regulates the kinetics of inflammatory and anti-inflammatory cytokines (Belkaid, Hand, 2014), synthesizes neurotransmitters (Yano *et al.*, 2015), and exerts strict control over energetic and endocrine metabolism (Cani *et al.*, 2007; Koh *et al.*, 2016). Developmental age, with its maximum biological plasticity, represents the prime temporal window in which this complex interaction is structured.

Intestinal eubiosis represents the cornerstone of the systemic homeostasis of the growing individual (Lozupone *et al.*, 2012).

Omics Sciences and Functional Profile of the Gut Microbiota

The quantum leap in the study of the gut microbiota has been made possible by the advent and spread of latest-generation omics sciences. Until a few years ago, knowledge of gut microorganisms was limited to classical microbiology techniques based on in vitro culture. Since over 80% of the bacterial species in the human gut consist of strict anaerobes that cannot be cultivated under standard conditions, the true entity and diversity of this ecosystem remained unexplored. Today, the metagenomic approach, through 16S ribosomal RNA gene sequencing and shotgun sequencing of the entire microbial genome, allows precise mapping of community

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taxonomy, identifying the phyla, classes, orders, families, genera, and species present (Turnbaugh *et al.*, 2009; Milani *et al.*, 2017). This methodological transition allows us to truly answer the question: "who is in the gut?". However, the true cognitive revolution has come from the possibility of functional analysis supported by transcriptomics, proteomics, and, most notably, metabolomics. Metabolomics studies the set of low molecular weight metabolites produced by bacterial metabolism starting from substrates introduced via diet or secreted by the host. These molecules constitute the true operational arm of the microbiota and the chemical means through which it exercises its remote directing action on the host's target organs (Sharon *et al.*, 2016). Among the most significant PNEI metabolites are short-chain fatty acids (SCFAs, such as acetate, propionate, and butyrate) (Koh *et al.*, 2016), secondary bile acids, indole derivatives (obtained from tryptophan catabolism), polyamines, and a vast array of microbially synthesized neurotransmitters, including gamma-aminobutyric acid (GABA), serotonin, dopamine, and acetylcholine (Cryan *et al.*, 2019). Metabolome analysis makes it possible to establish a direct causal link between the biochemical activity of the microbiota and the physiological or pathological responses of the host, revealing the fine mechanisms of infant homeostasis by studying "what that particular gut bacterial community does" (Milani *et al.*, 2017).

Developmental Age as a Programming Window and the First 1000 Days

David Barker's concept of "fetal and neonatal programming" (Early Life Programming) postulates that the environmental, nutritional, metabolic, toxicological, and psychological stimuli acting during the very early phases of pre- and postnatal development are capable of permanently shaping the structure, physiology, and gene expression of the organism, determining susceptibility to chronic diseases in adulthood (Barker, 1998). Developmental age must therefore not be understood as a mere phase of volumetric and ponderal growth, but rather as the period of maximum biological plasticity of the human being, a critical window in which the individual's "blueprint" is written. International scientific literature identifies this crucial interval in the so-called "first 1000 days" (Wopereis *et al.*, 2014), a time frame spanning from conception to the end of the second year of life. Recently, the PNEI vision has extended this conceptual horizon to the "first 6000 days", including childhood and adolescence, phases marked by further and extremely fine neuronal, endocrine, and immune reorganization, such as late synaptogenesis, cerebral pruning, and the maturation of mesocorticolimbic reward circuits during puberty (Cryan *et al.*, 2019). The gut microbiota fits perfectly into this programming process

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(Tamburini *et al.*, 2016). The development and maturation of the microbial ecosystem occur in parallel with the maturation of the child's biological systems (Koenig *et al.*, 2011). It has been widely demonstrated that the developmental trajectories of the immune system, the central nervous system, and the metabolic axis are intimately guided by signals originating from the microbiota (Gensollen *et al.*, 2016; Foster *et al.*, 2017). If microbial colonization occurs according to physiological timing and modalities, the organism receives the correct inputs to develop immune tolerance, neuroendocrine stability, and metabolic flexibility. Conversely, an alteration of the normal developmental trajectory of the microbiota in this critical temporal window leads to a deviation of the biological program (mis-programming), laying the groundwork for the long-term development of allergies, autoimmune diseases, obesity, metabolic syndrome, and neurobehavioral disorders (Arrieta *et al.*, 2015; Subramanian *et al.*, 2014).

Ecological Succession of the Microbiota in the First 36 Months of Life

The colonization of the newborn's gut follows a rigorous ecological succession that is completed between 30 and 36 months of life (Bäckhed *et al.*, 2015) (Tabella 1). At the end of this pathway, the child's microbiota reaches a complexity, richness, and stability comparable to that of an adult, defining the Community State Type (CST) or definitive enterotype of the subject (Turnbaugh *et al.*, 2009; Lozupone *et al.*, 2012). Although the question of the existence of an intrauterine fetal microbiota is still open, it is undisputed that the moment of birth represents the true microbial "big bang" (Dominguez-Bello *et al.*, 2010). In the first hours and days of postnatal life, the newborn's gut – characterized by a luminal environment with a high redox potential due to the presence of residual oxygen – is colonized by facultative anaerobic microorganisms, mainly enterobacteria such as *Escherichia coli* and streptococci. These pioneers consume free oxygen, modifying the luminal microenvironment and creating the conditions of strict anaerobiosis necessary for the establishment of subsequent colonizers drawn from the maternal environment (Milani *et al.*, 2017). Starting from the first weeks of life and throughout the period of exclusive breastfeeding, the microbiota of the healthy and eubiotic infant undergoes a drastic reduction in overall diversity, being literally dominated by a single phylum: *Actinobacteria*, represented for over 50-70% by the genus *Bifidobacterium* (in particular *B. infantis*, *B. bifidum*, and *B. breve*) (Milani *et al.*, 2017). This bifidobacterial dominance is a fundamental biological characteristic of the human species, phylogenetically selected for the utilization of human milk oligosaccharides (HMOs) (Bode, 2012). Bifidobacteria metabolize HMOs producing large quantities

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of acetate and lactate, molecules that acidify the intestinal luminal pH, exercising a natural barrier action against the establishment of pathogens (such as *Clostridioides difficile* and *Salmonella*) and guiding the early phases of education of the mucosa-associated lymphoid tissue (GALT) (Round, Mazmanian, 2009). The subsequent ecological turning point coincides with weaning and the introduction of solid foods and dietary fiber (Koenig *et al.*, 2011). The transition to a diversified diet deprives the microbiota of the exclusive substrate represented by milk and introduces complex carbohydrates indigestible by the host. This radical change promotes the proliferation of taxa specialized in the degradation of plant fiber, resulting in a progressive relative decline of bifidobacteria and a massive increase in the phyla *Firmicutes* (represented by butyrate-producing genera such as *Faecalibacterium* and *Roseburia*) and *Bacteroidetes* (Bäckhed *et al.*, 2015; Koh *et al.*, 2016). Around three years of life, the ecosystem stabilizes on the typical adult profile, characterized by a dynamic balance composed approximately of 45% *Firmicutes*, 45% *Bacteroidetes*, and the remaining 10% divided among *Actinobacteria*, *Verrucomicrobia* (e.g., *Akkermansia muciniphila*), and minor taxa (Turnbaugh *et al.*, 2009; Lozupone *et al.*, 2012).

Tab. 1. Ecological Succession of Intestinal Colonization from 0 to 36 Months

Developmental Phase	*Temporal Epoch*	*Dominant Phyla / Taxa*	*Functional and PNEI Characteristics*
Immediate Neonatal Period	0 - 7 days	*Proteobacteria* (*Enterobacteriaceae*, *E. coli*), *Streptococcaceae*	Consumption of luminal oxygen, shift toward anaerobiosis. Early interactions with innate immunity (Milani *et al.*, 2017).
Exclusive Breastfeeding	1 wk - 6 months	*Actinobacteria* (genus *Bifidobacterium* > 50-70%)	Selective consumption of HMOs, production of acetate and lactate, luminal acidification, anti-pathogen protection (Bode, 2012; Milani *et al.*, 2017).
Weaning	6 months - 24 months	Gradual expansion of *Firmicutes* and *Bacteroidetes*	Adaptation to complex dietary carbohydrates, increase in ecological alpha-diversity (Koenig *et al.*, 2011; Bäckhed *et al.*, 2015).
Adult Maturation and Eubiosis	24 - 36 months	*Firmicutes* (45%), *Bacteroidetes* (45%), *Actinobacteria*/Others 10%	Stabilization of the Community State Type (CST). Massive production of SCFAs (Turnbaugh *et al.*, 2009; Bäckhed *et al.*, 2015).

Note. *Dynamic of colonization and ecological succession associated with metabolic and PNEI regulation functions in the first 36 months of life. HMOs (Human Milk Oligosaccharides); SCFAs (Short Chain Fatty Acids); CST (Community State Type).*

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Early Dysbiosis and Shift toward Gram-Negativity

The delicate process of microbial ecological succession is highly vulnerable and can be perturbed by multiple environmental, iatrogenic, and relational factors, collectively defined as "drivers of early dysbiosis" (Tamburini *et al.*, 2016). The presence of one or more of these drivers alters the physiological trajectory of colonization, inducing a state of lasting imbalance that impacts the child's global programming (Arrieta *et al.*, 2015). The main drivers documented by clinical research include:

- **Cesarean Delivery:** The infant born via vaginal delivery is appropriately colonized by maternal vaginal and fecal microbiota (rich in *Lactobacillus*, *Bifidobacterium*, and *Bacteroides*). Conversely, the infant born via cesarean section is colonized by environmental and hospital skin-derived microorganisms (such as *Staphylococcus*, *Streptococcus*, and *Propionibacterium*), showing a severe delay in the establishment of protective bifidobacteria and a reduced overall biodiversity (Dominguez-Bello *et al.*, 2010; Neu, Rushing, 2011).
- **Prematurity:** Preterm infants present an anatomically and physiologically immature gastrointestinal tract, with a hyper-permeable epithelial barrier and hypomotility. Prolonged hospital stay in Neonatal Intensive Care Units (NICUs), massive exposure to sterile environments, and artificial nutrition hinder normal ecological colonization, favoring the engraftment of opportunistic and endotoxic pathogenic strains and delaying GALT maturation (Milani *et al.*, 2017).
- **Formula Feeding:** Artificial formulas lack the complex bioactive fraction of human milk, including immunoglobulins (secretory IgA), lactoferrin, lysozymes, immunomodulatory cytokines, and above all, the extraordinary variety of HMOs. In the absence of HMOs, the selective substrate for bifidobacterial dominance is lost, leading to premature and unbalanced growth of species typical of the adult microbiota or, worse, potentially pathogenic species (Bode, 2012).
- **Early Antibiotic Therapy:** Intrapartum exposure (maternal antibiotic prophylaxis for group B Streptococcus screening) or direct postnatal exposure to broad-spectrum antibiotics in the first months of life exerts a devastating effect on the intestinal ecosystem. Antibiotics act by wiping out entire populations of sensitive eubiotic bacteria (primarily bifidobacteria) and leaving empty ecological niches that are rapidly occupied by resistant microorganisms or Gram-negative opportunistic pathogens (Arrieta *et al.*, 2015).
- **Early Life Stress (ELS) and Early Life Adversity (ELA):** Stress perceived by the infant or toddler, resulting from maternal deprivation, non-sintonics caregiving, trauma, or alterations in the parental brain, translates biologically into chronic

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hyperactivation of the HPA axis with hypersecretion of cortisol and catecholamines (O'Mahony *et al.*, 2009; Foster *et al.*, 2017). These stress hormones directly alter intestinal motility, reduce mucus production by Goblet cells, and increase the permeability of the epithelial barrier. Furthermore, catecholamines released into the intestinal lumen can act as true molecular growth factors for specific pathogenic bacterial species, modulating the expression of their virulence genes (Foster *et al.*, 2017).

The consequence common to all these dysbiosis drivers is the loss of balance and microbial biodiversity, associated with a shift toward Gram-negativity (Arrieta *et al.*, 2015). Under eubiotic conditions, the infant gut is dominated by Gram-positive bacteria (Milani *et al.*, 2017). When dysbiotic drivers intervene, a massive proliferation of Gram-negative bacteria is observed, belonging primarily to the *Proteobacteria* phylum (such as *Enterobacteriaceae*, *Klebsiella*, *Pseudomonas*, *Serratia*) (Arrieta *et al.*, 2015; Milani *et al.*, 2017). The outer membrane of Gram-negative bacteria is composed for 80% of lipopolysaccharide (LPS), a potent pathogen-associated molecular pattern (PAMP) endowed with extremely high endotoxic and inflammatory activity (Cani *et al.*, 2007). This molecular imbalance represents the *primum movens* of the disruption of infant homeostasis and the activation of systemic inflammatory pathways (Belkaid, Hand, 2014).

Epithelial Cross-Talk, Immune Mechanisms, and Metabolic Endotoxemia

To understand the microbiota as a PNEI organ, it is necessary to examine the biochemical interface where the interaction between microbes and the host takes place: the intestinal epithelial barrier (Belkaid, Hand, 2014). In a healthy wall, enterocytes are tightly welded together by junctional protein complexes known as Tight Junctions (consisting of occludin, zonula occludens-1, claudins), which seal the intercellular space, preventing the uncontrolled paracellular passage of macromolecules, food antigens, and microorganisms. Above the enterocytes lies a continuous layer of hydrophilic mucus, synthesized and secreted by Goblet cells. This mucus acts as a physical and chemical protective shield, within which the eubiotic microbiota resides safely without coming into direct contact with the cellular plasma membrane (Round, Mazmanian, 2009). Below the intestinal epithelium lies the lamina propria, a loose connective tissue that hosts the largest concentration of immune cells in the entire organism, the GALT (Gut-Associated Lymphoid Tissue) (Belkaid, Hand, 2014; Gensollen *et al.*, 2016). The innate immune system and the enterocytes themselves are equipped with specialized pattern recognition receptors, called PRRs (Pattern Recognition Receptors), among which Toll-Like Receptors (specifically TLR-4) stand

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out (Gensollen *et al.*, 2016). Immune cells constantly "sense" the presence of microbes through continuous molecular cross-talk (Round, Mazmanian, 2009). Under eubiosis, weak and tolerogenic signals from bifidobacteria and lactobacilli stimulate dendritic cells to produce regulatory cytokines, such as interleukin-10 (IL-10) and Transforming Growth Factor-beta (TGF-beta), which promote the differentiation of T lymphocytes into regulatory T cells (Treg) (Belkaid, Hand, 2014). Tregs maintain immunological tolerance and stimulate isotype switching and the subsequent synthesis and secretion of secretory Immunoglobulins A (sIgA) into the intestinal lumen, consolidating mucosal protection without triggering severe inflammatory responses (Round, Mazmanian, 2009; Gensollen *et al.*, 2016).

When early dysbiosis with a shift toward Gram-negativity occurs, this delicate tolerance mechanism breaks down (Arrieta *et al.*, 2015). The overproduction of lipopolysaccharide (LPS) massively saturates and activates the TLR-4 receptor expressed on the surface of enterocytes and innate immune cells (Cani *et al.*, 2007). TLR-4 engagement triggers an intracellular signaling cascade mediated by the adapter protein MyD88, culminating in the nuclear translocation of the transcription factor NFKB (Nuclear Factor kappa B). The activation of NFKB induces gene expression and the subsequent release of a storm of pro-inflammatory cytokines, such as Tumor Necrosis Factor-alpha (TNF-alpha), interleukin-1 beta (IL-1beta), and interleukin-6 (IL-6), activating the intracellular inflammasome (Cani *et al.*, 2007; Belkaid, Hand, 2014). These inflammatory cytokines exert a direct cytotoxic effect on Tight Junction proteins, causing their disorganization, dephosphorylation, and degradation. This destabilizes the integrity of the intestinal barrier, configuring the pathophysiological condition known as "increased intestinal permeability syndrome" or Leaky Gut Syndrome. The loss of barrier function allows the massive paracellular passage of bacterial fragments, food antigens, and, above all, LPS itself from the intestinal lumen directly into the bloodstream and lymphatic circulation (Cani *et al.*, 2007). This phenomenon, termed "metabolic endotoxemia", distributes the inflammatory stimulus systemically (Cani *et al.*, 2007). Circulating LPS, by binding to TLR-4 receptors expressed in vascular beds and distant organs (liver, adipose tissue, blood vessels), fuels a state of low-grade chronic inflammation (Low-Grade Chronic Inflammation), which acts as a constant element of disturbance for all integrated biological systems of the growing host (Cani *et al.*, 2007).

The Gut-Microbiota-Brain Axis and the Impact on Neurodevelopment

One of the most fascinating and clinically relevant bidirectional axes from a PNEI perspective is the gut-microbiota-brain axis (Gut-Microbiota-Brain Axis)

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(Cryan *et al.*, 2019). During developmental age, the brain undergoes an extraordinarily rapid and complex phase of development. If at birth the newborn's brain mass represents about one-third of that of an adult, by the second year of life it reaches 80-90% of its final volume, showing unmatched kinetic speed in connectome formation, synaptogenesis, myelination, and synaptic pruning processes (Diaz Heijtz *et al.*, 2011; Sharon *et al.*, 2016). The gut microbiota acts as the occult director of this complex neurological maturation through three main anatomical and biochemical communication pathways (Cryan *et al.*, 2019; Sharon *et al.*, 2016).

- **The Neural Pathway (The Vagus Nerve):** The vagus nerve constitutes the main direct neuroanatomical communication highway between the enteric nervous system (ENS) and the central nervous system (CNS). Vagal afferent fibers terminate at the intestinal mucosa, where they are positioned in immediate proximity to enteroendocrine and enterochromaffin cells. The microbiota, through the production of metabolites and neurotransmitters, electrically stimulates vagal receptors, sending instant signals to the nucleus of the solitary tract in the brainstem, from which information propagates toward limbic and cortical structures responsible for emotional, behavioral, and cognitive regulation (Foster *et al.*, 2017; Cryan *et al.*, 2019).
- **The Endocrine Pathway and Neurotransmitters:** The gut microbiota possesses the enzymatic machinery necessary for the *de novo* synthesis of neurotransmitters or for the regulation of the availability of their amino acid precursors in the host (Cryan *et al.*, 2019). Over 90% of the body's total serotonin (5-HT) is synthesized at the intestinal level by enterochromaffin cells, a process finely regulated by short-chain fatty acids (SCFAs) produced by bacteria (Yano *et al.*, 2015). Although peripheral serotonin is unable to cross the blood-brain barrier (BBB), it regulates intestinal motility and vagal signaling (Yano *et al.*, 2015). Furthermore, the microbiota modulates the systemic availability of tryptophan, the amino acid precursor of serotonin, directing it toward neurotransmitter synthesis or the kynurenine metabolic pathway; early dysbiosis shifts metabolism toward the kynurenine pathway, generating neurotoxic metabolites (such as quinolinic acid) associated with neuroinflammation and behavioral alterations (Clarke *et al.*, 2013; Cryan *et al.*, 2019). Eubiotic bacterial genera such as *Bifidobacterium* and *Lactobacillus* are also known for their ability to secrete high concentrations of GABA, the primary inhibitory neurotransmitter of the CNS, essential for countering excitotoxicity, regulating anxiety states, and stabilizing neuronal excitability (Cryan *et al.*, 2019).
- **The Immune Pathway and Microglial Function:** This represents the biochemical connection pathway most sensitive to dysbiotic states (Erny *et al.*, 2015). Microglia consist of the resident immune cells of the central nervous system, re-

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sponsible not only for anti-infective defense, but above all for synaptic shaping: microglia actively eliminate redundant or non-functioning synapses, allowing the correct configuration of cognitive and emotional neuronal circuits (Erny *et al.*, 2015). Pioneering studies on germ-free animal models (microbe-free) have unequivocally demonstrated that in the absence of a physiological gut microbiota or in the presence of severe early dysbiosis, microglia remain structurally immature, poorly distributed, and functionally dysfunctional (Erny *et al.*, 2015; Cryan *et al.*, 2019). Healthy microbial metabolites, particularly butyrate and propionate, are capable of crossing the blood-brain barrier and guiding the gene and morphological maturation of microglia (Erny *et al.*, 2015; Koh *et al.*, 2016). Conversely, metabolic endotoxemia from LPS activates microglia in a pro-inflammatory sense (M1 phenotype), triggering a silent neuroinflammation process that alters synaptic pruning and heavily interferes with neurodevelopmental trajectories, increasing the biological risk of onset of autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), and learning disorders (LD) (Cani *et al.*, 2007; Erny *et al.*, 2015; Sampson *et al.*, 2016).

Metabolic Regulation, Endocrine Homeostasis, and Epigenetic Markings

The directing action of the gut microbiota extends capillarically to the metabolic and endocrine sphere of the growing subject (Cani *et al.*, 2007; Koh *et al.*, 2016). Hormonal homeostasis in developmental age is a fundamental prerequisite to guarantee not only linear staturo-ponderal growth, but also the correct calibration of circadian rhythms (Bass, Takahashi, 2010; Codoñer-Franch, Gombert, 2018), the neuroendocrine response to stress (Sudo *et al.*, 2004), and future pubertal maturation. The microbiota intervenes in this area by acting as a true "metabolic transducer" (De Vadder *et al.*, 2014; Koh *et al.*, 2016). Through the production of SCFAs, it interacts with specific G protein-coupled receptors (GPR41 and GPR43) expressed on colonic enteroendocrine cells, stimulating the secretion of key peptide hormones such as Glucagon-Like Peptide-1 (GLP-1) and Peptide YY (PYY), central regulators of satiety, insulin sensitivity, and gastric motility (Koh *et al.*, 2016).

Furthermore, the gut microbiota possesses an active metabolic role in the biochemistry and recirculation of systemic hormones. It interferes with the thyroid axis, influencing the absorption of essential micronutrients (iodine, selenium, zinc) and regulating peripheral deiodinase activity; it modulates the adrenal axis, intervening in the kinetics of cortisol degradation and clearance and modifying the glucocorticoid sensitivity set-point (Sudo *et al.*, 2004); finally, it participates in the enterohepatic circulation of estrogens and sex hormones through the enzymatic activ-

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ity of microbial beta-glucuronidase (the so-called "estrobolome"), regulating the fraction of active hormones reabsorbed into circulation and conditioning the timing of adrenarche and gonadarche (Cryan *et al.*, 2019).

The deepest and most lasting molecular mechanism through which the microbiota and its metabolome shape endocrine-metabolic homeostasis is, however, represented by epigenetics (Stilling *et al.*, 2014). Epigenetics encompasses the set of heritable modifications that alter gene expression without changing the primary nucleotide sequence of DNA (Stilling *et al.*, 2014). It is estimated that human health and aging are determined 75% by epigenetic processes influenced by environment and lifestyle (the exposome), and only 25% by inherited genetics (Bottaccioli, Bottaccioli, 2020). The main epigenetic mechanisms include DNA methylation (which typically silences gene expression), histone acetylation/deacetylation (which opens or closes chromatin, regulating RNA polymerase accessibility to gene promoters), and post-transcriptional micro-RNA synthesis (Stilling *et al.*, 2014).

Metabolites produced by a eubiotic microbiota act as powerful epigenetic modifiers (Stilling *et al.*, 2014). Butyrate, for example, is a known and potent natural inhibitor of histone deacetylases (HDACi) (Koh *et al.*, 2016). By inhibiting HDACs, butyrate promotes histone hyperacetylation, keeping chromatin in an open and accessible conformation (euchromatin) that favors the transcription of protective genes, such as tumor suppressor genes, genes involved in metabolic stability, and neurotrophic factors like BDNF (Brain-Derived Neurotrophic Factor), essential for neuronal plasticity and learning (Stilling *et al.*, 2014). Bifidobacteria, through acetate production and the modulation of carbon metabolism and methyl groups (folate pathway), provide the biochemical substrates necessary for DNA methylation processes (Milani *et al.*, 2017). When early dysbiosis occurs with alteration of the microbial metabolome, incorrect epigenetic markings are generated in the nucleus of the growing subject's cells (Stilling *et al.*, 2014). This epigenetic "writing error" can stably silence crucial hormonal receptors or stably activate pro-inflammatory gene promoters, determining biological mis-programming that exposes the child to an exponentially higher risk of developing chronic-degenerative pathologies (type 2 diabetes, atherosclerosis, neurodegeneration, inflammatory bowel diseases) in adulthood (Barker, 1998; Cani *et al.*, 2007).

Clinical Strategies of Primary Prevention and Integrated Therapeutic Intervention

Understanding the gut microbiota as a PNEI director of developmental age requires the development of strategies for systemic and early primary prevention, to be implemented starting from the preconception era (Bottaccioli, Bottaccioli, 2020).

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Since the maternal metabolome regulates and directs fetal programming through the transplacental passage of metabolites and immunomodulatory signals (Barker, 1998; Vuong *et al.*, 2020), caring for the eubiosis, diet, and lifestyle of the pregnant woman represents the first and indispensable preventive medical act. A maternal diet rich in prebiotic fiber (Mediterranean diet), associated with psychological stress control and the reduction of chemical-physical environmental contaminants, favors the structuring of a healthy maternal microbiota, optimizing fetal programming and reducing the risk of adverse neonatal outcomes (Barker, 1998). In the postnatal period, the pediatrician's primary goal must be the protection of the ecological trajectory of the first 30-36 months, minimizing iatrogenic drivers of dysbiosis (Wopereis *et al.*, 2014; Bäckhed *et al.*, 2015). This translates into encouraging vaginal delivery where possible, strenuously promoting exclusive breastfeeding for the first 6 months, and applying criteria of extreme clinical appropriateness and restriction in the use of empirical antibiotic therapy (Dominguez-Bello *et al.*, 2010; Bode, 2012; Arrieta *et al.*, 2015). Should the dysbiotic event prove inevitable (e.g., medically necessary cesarean section, prematurity, maternal hypogalactia, or absolute necessity for antibiotic therapy), it is imperative to intervene promptly through integrated and targeted nutraceutical support, based on the rational use of biotics (Hill *et al.*, 2014; Gibson *et al.*, 2017; Salminen *et al.*, 2021):

- **Prebiotics:** Non-viable substrates selectively utilized by host microorganisms conferring a health benefit. In pediatrics, the use of oligosaccharides such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), capable of partially mimicking the action of human milk oligosaccharides (HMOs), favors the selective expansion of endogenous bifidobacteria and supports luminal acidification, improving barrier function (Gibson *et al.*, 2017).
- **Probiotics:** Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Prescription must not be generic, but strictly guided by strain specificity and clinical evidence (Hill *et al.*, 2014).
- **Synbiotics:** Synergistic formulations combining a live probiotic microorganism and a prebiotic substrate in the same product, optimizing the survival, transit, and engraftment of the administered strain in the host's intestinal lumen (Gibson *et al.*, 2017).
- **Postbiotics:** In accordance with the most recent international scientific consensus definitions (ISAPP, International Scientific Association for Probiotics and Prebiotics), postbiotics consist of preparations of inanimate microorganisms (inactivated, thermally killed, or tyndallized) and/or their structural cellular components (e.g., teichoic acids, peptidoglycans, membrane fragments, surface proteins) that confer a health benefit on the host, whether or not associated with their metabolites (Salminen *et al.*, 2021).

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Conclusions and Future Perspectives

The gut microbiota has demonstrated beyond a reasonable doubt that it exercises an integrated regulatory role in the biological choreography of developmental age (Cryan *et al.*, 2019). Through the production of a dynamic metabolome and the generation of bidirectional biochemical signals running along PNEI axes, this microscopic organ modulates the maturation of the immune system, calibrates endocrine-metabolic homeostasis, and directs the child's neurodevelopmental trajectories, impressing stable epigenetic markings that will condition the individual's health for the rest of their existence (Cani *et al.*, 2007; Stilling *et al.*, 2014; Cryan *et al.*, 2019). The preservation of intestinal eubiosis in the early years of life must no longer be considered complementary or optional, but a fundamental clinical and preventive priority for the public health of the future.

Funding: This research received no external funding. Conflicts of Interest: The authors declare no conflict of interest. All authors have read and agreed to the published version of the manuscript.

References

Arrieta M.C., Stiemsma L.T., Dimitriu P.A., Thorson L., Russell S., & Yurist-Doutsch S. (2015). Early infancy microbial dysbiosis is associated with increased risk of asthma. *Science Translational Medicine*, 7(307), 307ra152. https://doi.org/10.1126/scitranslmed.aab2271

Bäckhed F., Roswall J., Peng Y., Feng Q., Jia H., & Kovatcheva-Datchary P. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host & Microbe*, 17(5), 690-703. https://doi.org/10.1016/j.chom.2015.04.004

Barker D.J.P. (1998). *Mothers, babies and health in later life*. Edinburgh: Churchill Livingstone.

Bass J., & Takahashi J.S. (2010). Circadian integration of metabolism and energetics. *Science*, 330(6009), 1349-1354. https://doi.org/10.1126/science.1195027

Belkaid Y., & Hand T.W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157(1), 121-141. https://doi.org/10.1016/j.cell.2014.03.011

Bode L. (2012). Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology*, 22(9), 1147-1162. https://doi.org/10.1093/glycob/cws074

Bottaccioli F., & Bottaccioli A.G. (2020). *Psiconeuroendocrinoimmunologia e scienza della cura integrata. Il manuale*. Milano: Edra.

Cani P.D., Amar J., Iglesias M.A., Poggi M., Knauf C., & Bastelica D. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, 56(7), 1761-1772. https://doi.org/10.2337/db06-1491

Clarke G., Grenham S., Scully P., Fitzgerald P., Moloney R.D., & Shanahan F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, 18(6), 666-673. https://doi.org/10.1038/mp.2012.77.

Codoñer-Franch P., & Gombert M. (2018). Circadian rhythms in the pathogenesis of gastrointestinal diseases. *World journal of gastroenterology*, 24(38), 4297-4303. https://doi.org/10.3748/wjg.v24.i38.4297

Cryan J.F., O'Riordan K.J., Cowan C.S.M., Sandhu K.V., Bastiaanssen T.F.S., & Boehme M. (2019). The microbiota-gut-brain axis. *Physiological reviews*, 99(4), 1877-2013. https://doi.org/10.1152/physrev.00018.2018.

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De Vadder F., Kovatcheva-Datchary P., Goncalves D., Vinera M., Zitoun C., & Duchampt A. (2014). Microbiota-generated metabolites promote metabolic benefits via gut gluconeogenesis. *Cell*, 156(1-2), 84-96. https://doi.org/10.1016/j.cell.2013.12.016.

Diaz Heijtz R., Wang S., Anuar F., Qian Y., Björkholm B., & Samuelsson A. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 108(7), 3047-3052. https://doi.org/10.1073/pnas.1010529108.

Dominguez-Bello M.G., Costello E.K., Contreras M., Magris M., Hidalgo G., & Fierer N. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, 107(26), 11971-11975. https://doi.org/10.1073/pnas.1002601107

Erny D., Angelis A.L., Jaitin D., Wieghofer P., Staszewski O., & David E. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, 18(7), 965-977. https://doi.org/10.1038/nn.4030

Foster J.A., Rinaman L., & Cryan J.F. (2017). Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiology of Stress*, 7, 124–136. https://doi.org/10.1016/j.ynstr.2017.03.001

Gensollen T., Iyer S.S., Kasper D.L., & Blumberg R.S. (2016). How colonization by microbiota in early life shapes the immune system. *Science*, 352(6285): 539-544. https://doi.org/10.1126/science.aad9378

Gibson G.R., Hutkins R., Sanders M.E., Prescott S.L., Reimer R.A., & Salminen S. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews. Gastroenterology & Hepatology*, 14(8): 491-502. https://doi.org/10.1038/nrgastro.2017.75

Hill C., Guarner F., Reid G., Gibson G.R., Merenstein D.J., & Pot B. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology & Hepatology*, 11(8), 506-514. https://doi.org/10.1038/nrgastro.2014.66

Koenig J.E., Spor A., Scalfone N., Fricker A.D., Stombaugh J., & Knight R. (2011). Succession of microbial communities in the infant gut and its relation to formatting the adult community. *Proceedings of the National Academy of Sciences of the United States of America*, 1(Suppl 1), 4578–4585. https://doi.org/10.1073/pnas.1000081107

Koh A., Vadder F., Kovatcheva-Datchary P., & Bäckhed F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell*, 165(6), 1332-1345. https://doi.org/10.1016/j.cell.2016.05.041

Lozupone C.A., Stombaugh J.I., Gordon J.I., Jansson J.K., & Knight R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, 489(7415), 220-230. https://doi.org/10.1038/nature11550.

Milani C., Duranti S., Bottacini F., Casey E., Turroni F., & Mahony J. (2017). The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews: MMBR*, 81(4), e00036-17. https://doi.org/10.1128/mmbr.00036-17

Neu J., & Rushing J. (2011). Cesarean versus vaginal delivery: Long-term infant outcomes and the hygiene hypothesis. *Clinics in Perinatology*, 38(2), 321-331. https://doi.org/10.1016/j.clp.2011.03.008

O'Mahony S.M., Marchesi S. JR, P C., C C., AM Q., & E.M.M. (2009). Early life stress alters behavior, immunity, and microbiota in rats: Implications for irritable bowel syndrome. *Biological Psychiatry*, 65(3), 263-267. https://doi.org/10.1016/j.biopsych.2008.06.026.

Ridaura V.K., Faith J.J., Rey F.E., Cheng J., Duncan A.E., & Kau A.L. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, 341(6150), 1241214. https://doi.org/10.1126/science.1241214

Round J.L., & Mazmanian S.K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature reviews. Immunology*, 9(5), 313-323. https://doi.org/10.1038/nri2515

Salminen S., Collado M.C., Endo A., Hill C., Lebeer S., & Quigley E.M.M. (2021). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition

PNEI review – ISSNe 2532-2826 – DOI: 10.3280/pnei2026oa23591
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and scope of postbiotics. *Nature Reviews. Gastroenterology & Hepatology*, 18(9), 649-667. https://doi.org/10.1038/s41575-021-00440-6

Sampson T.R., Debelius J.W., Thron T., Janssen S., Shastri G.G., & Ilhan Z.E. (2016). Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*, 167(6), 1469-1480. https://doi.org/10.1016/j.cell.2016.11.018

Sharon G., Sampson T.R., Geschwind D.H., & Mazmanian S.K. (2016). The central nervous system and the gut microbiome. *Cell*, 167(4), 915-932. https://doi.org/10.1016/j.cell.2016.10.027

Stilling R. M., Dinan T. G. & Cryan, J. F. (2014). Microbial genes, brain & behaviour – epigenetic regulation of the gut–brain axis**.** *Genes, Brain and Behavior*, 13(1), 69–86. https://doi.org/10.1111/gbb.12109.

Subramanian S., Huq S., Yatsunenko T., Haque R., Mahfuz M., & Alam M.A. (2014). Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature*, 510(7505), 417-421. https://doi.org/10.1038/nature13421.

Sudo N., Chida Y., Aiba Y., Sonoda J., Oyama N., & Yu X.N. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, 558(Pt 1), 263-275. https://doi.org/10.1113/jphysiol.2004.063388

Tamburini S., Shen N., Wu H.C., & Clemente J.C. (2016). The microbiome in early life: Implications for health outcomes. *Nature Medicine*, 22(7), 713-722. https://doi.org/10.1038/nm.4142

Turnbaugh P.J., Hamady M., Yatsunenko T., Cantarel B.L., Duncan A., & Ley R.E. (2009). A core gut microbiome in obese and lean twins. *Nature*, 457(7228), 480-484. https://doi.org/10.1038/nature07540

Vuong H.E., Pronovost G.N., Williams D.W., Coley E.J.L., Siegler E.L., & Carey A. (2020). The maternal microbiome modulates fetal neurodevelopment in mice. *Nature*, 586(7828), 281-286. https://doi.org/10.1038/s41586-020-2745-3.

Wopereis H., Oozeer R., Knipping K., Kraneveld A.D., & Garssen J. (2014). The first thousand days - intestinal microbiology of early life: Establishing a symbiosis. *Pediatric Allergy and Immunology: Official Publication of the European Society of Pediatric Allergy and Immunology*, 25(5), 428-438. https://doi.org/10.1111/pai.12232

Yano J.M., Yu K., Donaldson G.P., Shastri G.G., Ann P., & Ma L. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, 161(2), 264-276. https://doi.org/10.1016/j.cell.2015.02.047

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