Abstract

There is mounting evidence that the gut microbiome has a profound impact on human health and disease. In this chapter, we discuss interactions between endocrine disrupting chemicals (EDCs) and gut microbiota. We consider how the gut microbiota, through cross-talk with the gut–liver and gut–brain axes, can mediate the outcome of EDC exposure. In addition, evidence is provided for the sex-specific, transgenerational effects of early EDC exposure, primarily through disruption of the vertical transfer of maternal gut microbiota. Furthermore, the potential role of gut microbiota dysbiosis in inflammatory, autoimmune, and anxiety-related...
17. INTRODUCTION

Human physiology has evolved over time in concert with the trillions of microbes that surround and reside in us. Human cells are outnumbered by the body’s microbial residents [1], of which the majority inhabit the intestinal epithelium and account for about 90% of the total microbes in/on the human body. The gastrointestinal (GI) tract harbors commensal microbes that depend on the host for survival without altering the host physiology, symbiotics that mutually share benefits with the host, and/or opportunistic pathogens that are inherently detrimental to the host’s health. These intestinal microorganisms are comprised of bacteria, viruses, fungi, and protozoa and are collectively called gut microbiota. The microbes, their genomes, and metabolites are termed the gut microbiome [2,3].

Gut microbiota influence host health throughout life, starting at or before birth [4,5]. Gut microbiota produce nutrient energy through metabolism of otherwise indigestible food sources, including fiber. These microbes of the intestine also produce essential vitamins, such as vitamin K, and metabolize bile, steroid hormones, drugs, and xenobiotics [6–9]. Gut microbes produce metabolites and ligands that are essential for the sensitization and maturation of the innate immune system [10]. Thus, alterations in gut microbiota have a profound impact on human health and disease. For example, dysbiosis in gut microbiota in humans and other mammals have been implicated in obesity, type 2 diabetes (T2D), cardiovascular diseases, anxiety, depression, inflammatory bowel conditions, and autoimmune disorders, including type 1 diabetes (T1D), asthma, and rheumatoid arthritis (Figure 17.1) [11–18].

17.2 EVOLUTION OF THE HUMAN GUT MICROBIOME

The community structure of microbes in the human gut has undergone significant changes over time [19]. Over the decades, substantial modifications have been introduced in food production and processing, lifestyle, family structure, and the external environment that could directly affect the gut microbiome. Cophylogeny analysis, which determines whether the host and the resident microbes share parallel associations in their evolution, suggests that microbes and host coevolve and are strongly affected by diet [20]. Profound changes in gut microbiota have been observed in Asian immigrants in the US, suggesting the impact of diet change and lifestyle on microbial health [21]. Omnivores, vegetarians, and vegans have distinct gut microbiome communities [22]. Moreover, reduced incidences of vaginal mode of delivery, decreases in the incidence and duration of breastfeeding, and increases in use of antibiotics (ABX) can all contribute to the loss of gut microbiota diversity over time [19,23]. The loss in microbiota diversity influences both horizontal transmission that affects changes over time on an individual or species level and vertical transmission, inducing transgenerational changes in the offspring’s gut microbiota.
The loss of gut microbiota diversity in humans has coincided with changes in certain disease manifestations and progression. One such loss over time has been observed for *Helicobacter pylori* (H. pylori), an indigenous microbial resident of the stomach. H. pylori is associated with gastric ulcers and gastric cancer [24]. ABX treatment for the eradication of H. pylori infection reduced the incidence of these gastric disorders, but also resulted in a population-level loss of this microbial strain. This loss of H. pylori has been irreversible, in particular through halting its vertical transmission. Coincidentally, with the disappearance of H. pylori, new disorders including esophageal cancer, childhood allergies, asthma, and obesity are on the rise [23]. This correlative evidence suggests that changes in lifestyle, food sources, and exaggerated use of ABX, all can result in a gradual loss of the gut microbiota diversity, in turn increasing incidence of chronic disorders [25].

**FIGURE 17.1 Effects of endocrine disrupting chemicals (EDCs) on human health.** Industrial pollution and pesticides contribute to EDC contamination of food and water, which then enter the food chain. Bisphenol A (BPA), another common EDC, can leach into food and beverages through packaging. Antibiotics (ABX), used in low levels in farm animals or through direct therapeutic use in humans, can act as EDCs. Major target organs, EDC-induced pathological changes, and the resulting disease outcomes are shown. ADHD, Attention-deficit hyperactivity disorder.

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**17.3 TIMELINE OF GUT MICROBIOME DEVELOPMENT**

Humans and mammals are initially colonized by maternal microbiota during birth, although some exposure may start in utero [4,26,27]. Gut microbiota in humans are dominated by *Bifidobacterium* in the first year and are stabilized by the age of 3 years, mostly resembling the adult microbiome [28]. Colonization of gut microbiota after birth is affected by maternal
diet via breast milk, sociocultural environment, geography, and genetic makeup giving rise to unique individual microbial signatures [28]. Alteration in these factors can disrupt the initial stabilization of the gut microbiota. For example, administration of prophylactic ABX to the mother during the intrapartum period can reduce the transfer of maternal vaginal microbes *Lactobacilli* and increase the ABX-resistant microbes such as *Escherichia coli*, *Citrobacter*, *Klebsiella*, and *Enterobacter* to infants [29]. Similarly, administration of the broad-spectrum ABX to neonates, or intrapartum ABX treatment to mothers, decreases gut microbiota diversity in infants, in particular by reducing *Bifidobacterium* and increasing *Enterococcus* [30].

The gut epithelium in newborns is permeable to macromolecules such as immunoglobulins present in breast milk [31], but the permeability is reduced within a few days after birth to prevent the invasion of pathogens [32]. Gut microbiota produce the fiber metabolites short-chain fatty acids (SCFAs), including butyrate, that exert local and systemic anti-inflammatory effects by attenuating the T-cell immune response to antigens [33,34]. In contrast, microbe-derived pathogenic molecules can induce potent immune responses. For example, lipopolysaccharide (LPS), a cell wall component of Gram-negative microbes, initiates the sensitization of the immune system and restricts pathogenic invasion [35]. Many intestinal cells also release antimicrobial proteins and immunoglobulin A in the gut mucosa to protect from infection [36].

A lack of initial sensitization of the developing immune system by bacterial metabolites is associated with autoimmune disorders. For example, exposure to an oligosaccharide derived from *Staphylococcus epidermis* induced a stronger arthritis response in germ-free rats (rats raised in sterile isolators and lack most microbes) than in conventional rats [37]. In humans, the exaggerated immune response in rheumatoid arthritis is usually associated with increased *Prevotella* species and activation of helper T cells 17 (Th17) located in the intestine [38]. T-cells provide protection against widespread acute and chronic infections. An impaired T-cell response is associated with severity of respiratory infections like coronavirus disease (COVID-19). COVID-19 is an acute respiratory disease caused by a coronavirus, SARS-CoV-2 [39]. COVID-19 is an ongoing global health crisis that has already claimed more than 1.3 million lives worldwide since first detected in Wuhan, China, in 2019 (https://coronavirus.jhu.edu/map.html). An aberrant elevation in T-cell response resulting in cytokine storm and ultimately T-cell exhaustion has been a hallmark of severity of the COVID-19 in people infected with the SARS-CoV-2 virus [40]. The primary route for the SARS-CoV-2 is through binding to angiotensin-converting enzyme II (ACE2) receptors present in alveolar epithelial cells [39]. Interestingly, intestinal epithelial cells also express ACE2 receptors, and are likely contributors to the GI symptoms, including nausea, vomiting, and diarrhea in COVID-19 patients [41]. Furthermore, gut microbiota composition significantly differs between COVID-19 positive patients and controls, with a decrease in *Faecalibacterium prausnitzii*, *Eubacterium rectale* and *Bifidobacteria* COVID-19 patients, suggesting a role for gut microbiota in COVID-19 infection [42,153].

Immune system development is further facilitated by gut microbiota and intestinal endocrine cells via the production of neurotransmitters. For example, serotonin (5-HT) is produced from enterochromaffin cells and many *Lactococci* species [43]. While 5-HT in the intestine is necessary for immune system development, a reduction in intestinal 5-HT is linked to inflammatory bowel disorders [44]. Dopamine and epinephrine are also produced by many *Bacilli* and are necessary for the maturation of immune system (Figure 17.2) [43,45,46]. Collectively, these data provide strong evidence for the role of
The gut microbiome is critical in neurodevelopment [5,47]. Gut microbes produce and metabolize neurotransmitters and their precursors that can act locally on the enteric system, or can cross the blood–brain barrier. For example, intestinal 5-HT acts through its 5HT-3 receptors in vagus nerve terminals [48]. Another neurotransmitter, acetylcholine, is synthesized by tuft cells, a type of enteroendocrine cells, and acts as an initial enteric signal to the developing brain (Figure 17.2) [49]. Many neurotransmitters can be synthesized by gut microbiota. For example, gamma-aminobutyric acid (GABA), produced by the Lactobacilli species L. plantarum, L. brevis, and L. Lactis [50], provides feedback signals to the developing nervous system. Intestinal hormones, including cholecystokinin and glucagon-like peptide, stimulate vagal afferents to regulate food intake [51]. The brain, in turn, provides feedback to the enteric system through the vagus nerve and neuroendocrine pathways. This bidirectional feedback between the gut and the brain is referred to as the gut–brain axis (Figure 17.2) [3,52]. Perturbations during early life, such as the mode of delivery, can negatively affect the gut–brain axis. For example, depletion of the maternal gut microbiome using ABX affected fetal neurodevelopment by reducing axon formations, which was rescued by recolonization of the pregnant dams with Clostridia-dominant spore-forming bacteria [53]. Moreover, caesarian-delivered mice show reduced gut microbiota and their regulation of enteroendocrine cells on the development of a healthy innate immune system.

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social interaction compared to vaginally delivered mice, which was corrected by cohousing with vaginally delivered mice or by direct replenishment of Bifidobacterium, a microbe that is depleted in caesarian-delivered mice [54]. In addition to the intestinal impact on brain development, the brain influences gut health. For example, inducing stress in rodents and primates increases exacerbation of colitis [55, 56]. Thus, normal development of the gut–brain axis is dependent on healthy gut microbiota.

17.3.1 Sex Differences in Gut Microbiome

Gut microbial communities differ between males and females across species, including mice, rats, and humans [57–60]. In a Spanish population, women showed a higher abundance of Bilophila, while men had increased abundances of Veillonella and Methanobrevibacter [57]. In another European population, gut microbiota in men had more abundance of Prevotella than in women [61]. Sex differences in gut microbial community also exist in mice, with males having increased Lachnospiraceae, Parabacteroides, and Proteobacteria compared to females [13, 62]. How these sex differences in gut microbiota contribute to differences in host health and disease are an important area of current investigation.

Sex steroid hormones, including estrogens, testosterone, and progesterone, profoundly influence microbiota in humans and rodents [62–67]. Urinary estrogens positively associate with a diverse gut microbiota community in postmenopausal women [68]. In rodents, depletion of gonadal hormones by ovariectomy alters gut microbial diversity, including the Bacteroidetes to Firmicutes ratio [69, 70]. Estrogens alter gut microbiota in obese leptin-deficient (ob/ob) female mice by reducing evenness and increasing S24-7 abundance [71]. Similarly, androgens alter gut microbiota such that testosterone administration to female neonatal rats attenuates gut microbial diversity during adulthood and increases the Firmicutes to Bacteroidetes ratio [59]. The transfer of gut microbiota from males to female nonobese diabetic mice attenuates the severity of diabetes in females in an androgen-dependent manner [62]. This sex-dependent effect of microbiota on the emergence and progression of diseases is referred to as microgenderome [72]. Hormones and synthetic hormone analogs can influence microgenderome by acting via estrogen receptors in the gut and throughout the body [73]. Thus, it is imperative to consider sex as an important variable in understanding the function of gut microbiota in health and disease.

17.4 ENDOCRINE DISRUPTING CHEMICALS

Gut microbiota can metabolize a variety of hormones, including estrogens [13, 74]. In addition, the gut microbial metabolites, SCFA, can alter neuropeptide production [75]. Through this regulation of the production of hormones, the highly heterogeneous yet strictly organized, gut microbial community serves as an endocrine organ [43]. Gut microbiota are profoundly affected by genetic background, hormones, and environment. Environmental pollutants ingested through food and water, or inhaled through the air, can alter gut microbial composition and abundance. Many such compounds
impact health by disrupting the endocrine system and are collectively called endocrine disrupting chemicals (EDCs). Classically, EDCs are defined as “an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action” [76]. Many EDCs act as hormone analogs by interacting with hormone receptors or by interfering with hormone production, metabolism, and function [77].

The effects of EDC on endocrine systems have been well described in the first edition of this book [78] and in many reviews (e.g., Refs. [77,79]. EDCs, such as heavy metals (e.g., lead, cadmium, and arsenic), pesticides (atrazine and dichlorodiphenyldichloroethylene (DDT)), plasticizers (bisphenol A (BPA), polyvinyl chloride (PVC), and phthalates)), synthetic hormones (ethinylestradiol (EE) and diethylstilbestrol (DES)), cookware (per- and polyfluoroalkyl substances (PFAS)), are abundant in the environment [77,79,80]. Many of these EDCs are widely used in food packaging, building supplies, cleaning and personal care products, and medical devices. Some compounds that are used as adjuvants in medicines to modulate drug release, delivery, and stability can also disrupt the human endocrine system [81,82].

Over the years, there has been increasing evidence for the role of gut microbiota in EDC metabolism. EDCs are easily absorbed, primarily in the distal region of the GI tract and then transported to the liver via the portal vein (Figure 17.2). The communication between the gut and liver is dependent on the biliary tract, portal vein, and systemic mediators, collectively referred to as the gut–liver axis [83]. The liver further influences the gut microbial ecosystem and gut morphology via the production of hormones, enzymes, and bile salts [154](Figure 17.2). EDCs, such as BPA and EE, exhibit widespread estrogenic effects acting on estrogen receptors [73,84,85]. These EDCs also undergo metabolic changes similar to estrogens in the liver and intestine. BPA and EE are conjugated with glucuronides in the liver by an enzyme UDP-glucuronosyltransferase [86,87], resulting in their inactivation and excretion via bile and urine [88,89]. However, the inactivated BPA and EE can be recycled to active metabolites following deconjugation by microbial enzymes, such as β-glucuronidase. These recycled EDCs then enter the circulation and continue to exert effects in the body (Figure 17.2) [90]. The deconjugating enzyme, β-glucuronidase, is widely produced by many resident gut microbes, including the families Bacteroidaceae, Bifidobacteriaceae, Clostridiaceae, Enterobacteriaceae, Lactobacillaceae, Ruminococcaceae, and Verrucomicrobiaceae [90]. For example, in postmenopausal women, fecal E2 levels are inversely correlated with β-glucuronidase, providing further evidence that E2 and estrogen-like compounds are recycled in the gut [7]. Gut microbiota are rich in additional enzymes, including cytochrome P450 (CYP450), carboxylesterases, carnobyl reductases, and carboxylesterases that metabolize nutrients, hormones, and xenobiotics by oxidation, reduction, and/or hydrolysis. These enzymes modify the actions or route of transport of hormones and hormone-like EDCs by conjugating them to form polar products to be excreted via bile or urine [91]. On the other hand, EDC exposure results in profound alterations in gut microbiota based on the type, dose, and timing of the EDC [92]. In summary, the gut–liver axis, primarily via hepatic and gut microbial enzymes, is important in EDC metabolism and inactivation.

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17.5 EFFECTS OF EDC EXPOSURE

17.5.1 Perinatal EDC Exposure

Exposure to EDCs during perinatal development in rodents causes epigenetic changes, reproductive and metabolic diseases, and anxiety and depression in adulthood [77, 93, 94]. To explore the possibility that these effects are accompanied by changes in gut microbiota, BPA or EE was administered to male and female mice just prior to mating and continued through pregnancy and breastfeeding in females [84]. In the male pups, BPA increased *Akkermansia, Methanobrevibacter,* and *Sutterella,* whereas EE increased Erysipelotrichaceae abundance. Compared to controls, BPA or EE exposed female pups had increased *Bifidobacterium,* a microbe with beneficial anti-inflammatory effects and a common constituent in many probiotic formulations. These findings suggest that *Bifidobacterium* may have compensatory effects against the EDC-induced inflammatory insults [84]. Perinatal BPA exposure also causes neutrophil infiltration and liver and colon inflammation in offspring. Similarly, in rabbits, perinatal BPA exposure from gestational day 15 to postnatal day 7 increases gut permeability, as measured by an increase in serum LPS [95]. BPA treatment in these rabbits was accompanied by a reduction in *Oscillospira* and Ruminococcaceae abundance, both taxa important for SCFA production. Furthermore, various species within the Ruminococcaceae family produce β-glucuronidases that metabolize phytoestrogens, suggesting bidirectional pathway between EDC and gut microbiota [96]. Taken together, these findings suggest that changes in gut microbiota and their metabolites mediate these BPA- and EE-induced impairments in health.

Chlorpyrifos, an organophosphorus insecticide, impairs gut function by increasing gut permeability and bacterial translocation to the liver in rats when exposed from gestation to weaning [97]. Moreover, chlorpyrifos impairs mucin production, TLR4 expression, and alters gut microbiota [97]. Another widely used pesticide, dichlorodiphenyltrichloroethane (DDT), was banned in the US in 1973, but its heavy use as a pesticide in many other countries is a continued concern. Moreover, detrimental effects of DDT exposure have been observed across generations [98]. Exposure to DDT of pregnant rats increased obesity and reproductive dysfunction in their third generation offspring. These effects were associated with differential gene methylation in germline cells [98]. DDT heavily disrupts the host endocrine system and gut microbiota homeostasis [6, 62]. In the human gut, DDT can be metabolized by *Eubacterium limosum,* which converts it to a less toxic compound, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDD) [99]. Therefore, the gut microbiota are potential therapeutic targets for mitigating the toxic effects of these EDCs.

Diethylhexyl phthalate (DEHP) is found in plastics and has been linked to autism spectrum disorders in children [100]. Exposure to DEHP in newborn infants from intravenous infusion tubes increases urinary DEHP metabolites. DEHP exposure in newborns alters gut microbiota by decreasing the abundance of the beneficial microbe *Bifidobacterium* and transiently increasing the opportunistic pathogens *Staphylococcus* and *Streptococcus* [101]. Considering the pivotal role of gut microbes on the development of the host immune system during the neonatal period, even a transient disruption in microbial structure could exert
profound and lasting effects. Taken together, these findings indicate that exposure to various EDC during the perinatal period can cause gut microbiota dysbiosis that have consequential effects on host health.

17.5.2 EDC Exposure Around Puberty

Puberty is a critical period of development that is characterized by reproductive matura-
tion and initiated by the activation of the hypothalamic—pituitary axis [102]. Perturbations to this axis due to changes in hormone production, function, and clearance can exert lasting changes in reproduction, brain plasticity, and metabolic and immune homeostasis [103,104]. Because EDCs are ubiquitously present in the environment, human exposure begins in the perinatal period and continues through puberty and adulthood [105]. There is a strong correlation between urinary phthalate levels and incidences of attention deficit hyper-
activity disorders in boys and girls [106]. In male rats, treatment with low doses of DEHP during gestation through lactation delayed puberty [107]. Similarly, prenatal BPA exposure in mice alters growth and sexual maturation in adolescence in both male and female offspring [108]. Many of the EDC effects could be linked to changes in the gut microbiota. In support of these effects of EDC exposure during adolescence, exposure of pregnant dams to BPA or EE until weaning (right before puberty) resulted in reduced Lactococcus and Desulfovibrio in female and male offspring, respectively. Similarly, DEHP administered orally to 6-week-old female mice increased the abundance of Lachnoclostridium and modestly decreased Akkermansia and Odoribacter (Figure 17.3) [109]. These findings suggest that EDCs can directly alter gut microbiota and disrupt the intestinal immune response and host physiology.

Nanoparticles, including silver nanoparticles (AgNP), arsenic, and iron, also alter gut function. Inorganic nanoparticles are introduced into our diets in various ways, such as during food packaging, food coloring, and water purification [110,111]. A low-dose cadmium administration in mice through drinking water starting right before mating and continuing in the offspring increased fat mass, triglycerides, leptin and free fatty acids into adulthood in males, but not females [112]. This increase in fat mass was preceded by changes in gut microbiota. In particular, Bifidobacterium and Preotella were decreased, whereas Sphingomo-

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nus, a microbe that can absorb cadmium, was increased in these mice. Fecal microbiota transplant (FMT) from cadmium-treated male mice increased fat mass in the recipient males, but was prevented by ABX treatment, confirming the role of gut microbiota in the cadmium-induced metabolic disorders in male mice [112].

Arsenic, a known carcinogen, acts as an EDC by disrupting steroid receptor-mediated gene regulation [113]. Arsenic is ingested through contaminated water and food (e.g., rice). Many rice-based infant cereals pose a serious public health concern due to the high levels of arsenic. The FDA tested various infant rice cereals and found that many samples exceeded the permitted arsenic dose in food (fda.gov, 2018 arsenic in food data). In mice, exposure to arsenic altered the Bacteroidetes to Firmicutes ratio and increased arginine metabolites in the circulation, possibly contributing to its carcinogenic effect [114]. While these studies provide insights into the impacts of EDCs, including heavy metal exposure in animal models,
studying their effects in humans is challenging. Thus, comparing animal models and in vitro studies with epidemiological data have been crucial to understanding the harmful impact of these compounds in humans [115].

17.5.3 EDC Exposure During Adulthood

Many epidemiological studies that have measured EDCs in bodily fluids reveal that humans are exposed to various EDCs throughout life. Gut microbiota are one of the first targets of EDC during passage through the GI tract. These findings suggest that microbial enzymes regulate the severity of the EDC effects through modulation of their metabolism.

In a study led by National Health and Nutrition Examination Survey, increased urinary levels of BPA were strongly associated with a higher mortality risk in men and women [116]. Animal models have been vital to identifying EDC action in multiple effector organs. In adult female mice, oral administration of BPA for 2 weeks exacerbated colitis, primarily by increasing inflammatory cytokines and decreasing serotonin metabolites in the colon [117]. Commonly used organophosphorus pesticides such as 1-methylcyclopropene (MCP) are linked to increased incidences of T2D in humans and mice [118]. Furthermore, FMT from MCP-treated mice induced diabetes in recipient mice by increasing acetate production.

FIGURE 17.3 Transgenerational effects of exposure to endocrine disrupting chemicals (EDCs). Pregnant mice exposed to EDCs result in profound changes in gut microbiota of offspring in a sex-dependent manner. Many of these changes contribute to the development of metabolic, inflammatory, and developmental disorders. ASD, Autism spectrum disorder.
and gluconeogenesis in the liver [118]. These studies provide compelling evidence for the role of gut microbiota in mediating the pathogenic effects of EDCs and support the development of gut microbiota as a therapeutic target for mitigating the effects of EDC exposure.

Although most EDCs are industrially synthesized chemicals or their derivatives, some naturally occurring compounds, such as phytoestrogens, also disrupt the endocrine system. Phytoestrogens are estrogens found in plants, including isoflavones. Though broadly regarded as beneficial, phytoestrogens can exert endocrine disrupting effects depending on the age, health status, and structure of an individual’s gut microbiota. In male mice, the common isoflavones, genistein and daidzein, acted similarly as E2 to reduce obesity and insulin resistance in chronic high-fat/high-sugar-diet fed groups. Metabolic rescue of these mice was associated with an increase in Akkermansia, Bifidobacterium, and Bacteroides and a decrease in Proteobacteria [13]. Isoflavones exert protective effects against metabolic disruption by altering the gut microbiota community and preventing the release of microbe-induced inflammatory ligands, such as LPS, and activation of the TLR4 [13]. Use of isoflavones in peri/postmenopausal women to compensate for the loss of ovarian estrogens is on the rise. In postmenopausal women, 1 month of isoflavone treatment increased the beneficial gut microbes Bifidobacterium and Faecalibacterium prausnitzii [155]. Interestingly, this increase in these microbes faded, or even further decreased, by 2 months, suggesting isoflavones as a therapeutic approach for managing short-term perimenopausal symptoms without any long-lasting effects on microbiota [155].

Absorption of heavy metals, including iron, is heavily regulated by gut microbiota. In support, germ-free mice have higher circulating levels of iron and iron transporter proteins than conventional mice [119]. A single dose of fecal microbiota transplant from conventional mice to germ-free mice reduced the levels of iron transporters, while ABX treatment increased them, suggesting that gut microbiota directly regulate iron homeostasis [119]. Another metal, AgNP, which is used in food packaging, cosmetics, medical supplies (e.g., masks and gowns), and children’s toys, has powerful antimicrobial and antifungal properties [120]. Despite the ubiquitous use of AgNP, regulatory guidelines pertaining to its toxicity have not been established. Hence, the effects of these nanoparticles need to be investigated and guidelines for their safe and appropriate use need to be developed. AgNP, arsenic, and iron alter gut permeability and bacterial and host genetic composition [110,111]. AgNP altered microbial phyla by increasing Bacteroidetes and decreasing Firmicutes [111]. Chronic exposure to AgNP in male and female rats impaired the gut immune response, including the downregulation of immunomodulatory genes TLR2, TLR4, and NOD2 and the mucosal barrier MUC3 [111]. These effects were sex-dependent, with a stronger downregulation of MUC3 and TLR4 in females. Thus, these heavy metal–induced impairments involve changes in gut microbiota in a sex-specific manner, indicating the need to consider sex as an important variable when implementing the prevention and treatment strategies against metal exposure.

17.6 ANTIBIOTICS AND ENDOCRINE DISRUPTION

Following the discovery of penicillin by Alexander Fleming in 1928, ABX have been ubiquitously used to treat a variety of infections and have served as life-saving drugs [121]. At the
same time, their overuse has given rise to ABX-resistant strains of microbes [122]. In addition to its therapeutic use, ABX have been used extensively in farm animals to accelerate their growth, resulting in a continuous source of low-dose ABX to humans through meat consumption. ABX are released into the environment through pharmaceutical waste, use in plants and aquatic animal farming, and human waste, causing ABX pollution [123]. A low concentration of ABX, that is not sufficient to inhibit the growth of all microbial strains, is present in sewage water and thus can contaminate drinking water [124]. These low-dose ABX can both inhibit the growth of susceptible microbial strains and induce the ABX-resistance of some other strains, thereby disrupting the gut microbial ecosystem [125]. Thus, it is critical to understand the effects of this continuous and prolonged exposure of different classes of ABX, primarily through the depletion of native gut microbiota in humans and animals [124].

ABX in therapeutic or subtherapeutic doses profoundly disrupt gut microbiota in humans and rodents [123,124]. The health consequences of the ABX treatment are dependent on the timing, duration, and the class of ABX exposure (reviewed in Ref. [123]). ABX can also interfere with hormone actions, primarily via the depletion of gut microbiota that secrete estrogen-metabolizing enzymes, including β-glucuronidase and cytochrome P450 3A polypeptide 4 (CYP3A4) [13,74,126]. CYP3A4A, which is expressed in the liver, intestine, and by many gut microbes, regulates the synthesis of estrogens, progestins, and testosterone and the metabolism of drugs and xenoestrogens [152]. Therefore, CYP3A4A may be an important regulator of ABX and other EDC effects [127,128]. A comprehensive analysis of data from 1963 to 2018 showed that ABX use was associated with a sevenfold increase in unintended pregnancies compared to women in control groups [129]. Moreover, treatment with ampicillin, erythromycin, or tetracycline altered plasma, urinary, and fecal estrogen levels in women receiving hormone therapy (HT) [130,131]. Rifampicin, an ABX commonly used for the treatment of tuberculosis, also attenuated plasma estrogens in women taking oral contraceptives [132,133]. In contrast, erythromycin and ketoconazole, a fungicide, increased plasma hormone levels in postmenopausal women receiving HT containing estradiol valerate and dienogest [132]. Consistent with the role of gut microbes on hormone metabolism, germ-free mice have a reduced absorption of estrogens in the caecum, compared to conventionally raised mice [134]. Therefore, a significant impact of ABX treatment needs to be attributed to the alteration in hormone metabolism when examining their effects. These findings further indicate that severity of the ABX-induced disruption varies during different developmental stages based on the particular hormonal pathways that are dominant during the period.

While ABX have not yet been classified as EDC in the existing literature, the evidence of significant endocrine disruption in humans and other animals provide a compelling reason to propose ABX as a new group of EDC. The profound effects of ABX on hormone metabolism is primarily mediated through their effects on gut microbiota [74,123]. A recent study using conventional and germ-free mice reveals that ABX affects the host primarily via the depletion of gut microbiota and resurgence of the ABX-resistant microbes, although ABX can also directly alter gene regulation of the host independent of the gut microbiota [135]. The diversity of the gut microbial community suggests a wide variety of, as yet, unidentified microbiome-dependent metabolic pathways for the endocrine disrupting effects of ABX. Thus, for the purpose of this review, classifying ABX as an EDC

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provides an additional platform for a thorough investigation of its impact on hormone-dependent disorders.

17.6.1 ABX Treatment and Diseases

The absence of gut microbiota in germ-free mice, or their depletion following ABX treatment, is associated with a higher incidence of autoimmune diseases, such as asthma and T1D [15,136,137]. ABX increase aberrant immune response via activation of Th2 immune cells, an important precursor to asthma development. Epidemiological findings reveal that an increase in ABX use in the first year of life is associated with an increase in asthma [137]. T1D is preceded by an autoimmune destruction of pancreatic beta cells. In humans, an increased incidence of T1D in children in recent decades has coincided with an increase in ABX exposure [138], suggesting early ABX treatment contributes to T1D. In mice, females are more prone to developing T1D than males [62,139]. A pulsed therapeutic dose of ABX, that mimics frequent ABX treatment courses in humans, increased the risk of T1D in male mice. However, in females, despite having worse symptoms than males, this same ABX treatment did not further increase T1D incidence, suggesting that gut microbiota and host sex interact to impact the immune system [15,62]. These microbe-dependent transfer on the immune system are strain-dependent, suggesting an interplay between genetics, sex, microbiota, and immunity.

A subtherapeutic dose of ABX treatment during puberty induces adiposity and alters hepatic glucose and lipid metabolism in male and female mice [141]. This subtherapeutic ABX treatment, which is an approved dose for use in farm animals, increased the glucose-dependent insulinotropic polypeptide and production of SCFAs, including acetate, propionate, and butyrate, in the gut and altered the hepatic gene expression involved in fatty acid and lipid metabolism [140]. Similarly, a chronic low-dose penicillin treatment, from birth or weaning to adulthood, increases fat mass in male mice fed a standard or high-fat diet. However, in females, this same ABX treatment induces fat mass gain only during high-fat diet feeding. Females are also protected from accompanying hyperinsulinemia in both diet conditions, suggesting that estrogens protect from the detrimental effects of diet and ABX. Interestingly, when the ABX was discontinued, the metabolic disruption persisted, although the microbiota returned to pretreatment state, suggesting a lasting detrimental effect of ABX on metabolic health [141]. In male mice fed a high-fat diet, treatment with ampicillin and neomycin attenuates gut permeability, endotoxemia, adipocyte hypertrophy, and inflammation [142]. Similarly, a 4-week-long ABX cocktail of ampicillin, metronidazole, neomycin, and vancomycin improves glucose tolerance, hepatic glucose, and bile metabolism and increases *A. muciniphila* [143]. Finally, ABX treatment affects social learning and anxiety response in rodents. A low dose of penicillin in pregnant dams that was continued until weaning increased aggression and decreased anxiety in male BALB/c mice pups, and decreased social behavior in both male and female offspring. These behavioral impairments were partially corrected by *L. rhamnous* in the ABX-treated dams [144]. These studies indicate that ABX-mediated metabolic and behavioral impairments involve the perturbation of the gut microbiota in a sex-specific manner, emphasizing the need for both males and females when investigating the effects of ABX on the host and offspring.
Gut microbial secretion can not only autoregulate microbiota function, they also facilitate communication with neighboring microbes, using signals called autoinducers \cite{135,145}. Autoinducers regulate gene expression in response to cell-population density change, a process termed quorum sensing, to protect the microbe from changes induced by the gut milieu \cite{146}. Autoinducers are one contributor to the ABX resistance of microbes such as *Staphylococcus aureus* and pose one of the biggest public health challenges \cite{147,148}. The identification of these compounds serves a great potential for the development of synthetic molecules that inhibit microbial autoinducers to reduce ABX-resistant infections \cite{148}.

Gut microbiota also rely on each other for essential nutrients. In drosophila, lactate produced by *L. plantarum* determines the survival of *Acetobacter pomurum*, a species essential for the regulation of host appetite \cite{149}. In recent years, compounds in antiseptics and disinfectants such as ammonium and phosphonium salts have been found to cause nontoxic quorum sensing disruption within marine bacteria *C. violaceum* and *V. harveyi* \cite{145}. Quorum sensing inhibitors impact the biofilm formation of cells, an essential mediator of cell-to-cell communications. Both marine bacterial strains *C. violaceum* and *V. harveyi* were used to determine whether violacein production, important for quorum sensing regulation in the host, is impacted by these salts \cite{145}. These findings suggest quorum sensing as an effective tool to understand the effects of EDC on microbial ecosystem. Quorum sensing signals can ultimately be targeted to mitigate the harmful effects of pathogens and exogenous compounds.

**17.8 TARGETING THE GUT MICROBIOME**

The major initiatives to reduce human exposure to EDC need to occur through implementation of stricter regulations and a public ban on the use of highly toxic chemicals. When the banning of these compounds is not possible, it is imperative to strictly monitor their use and raise public awareness and education by providing clear product labels with known and potential health hazards \cite{150}. Moreover, future investigations should consider the cumulative and transgenerational effects of EDCs throughout communities. Different mitigating strategies that target the metabolism or function of EDCs are currently being explored. Given that gut microbiota significantly alter EDC metabolism, EDC-metabolizing functions should be studied as therapeutic targets. For example, β-glucuronidase inhibitors have been identified as a target to inhibit the production of active metabolites of EDCs and expedite removal from the body \cite{90}. Some inhibitors use β-glucuronidase-dependent deconjugation to self-activate, which can then induce mild diarrhea for faster removal of the luminal contents, including EDCs \cite{90}. With microbial genes exceeding 10 times that of total human genes, there is strong potential for the identification of microbial enzymes that can be used as detoxifiers against EDCs \cite{151}. These approaches can be used to develop personalized microbiome therapies based on the exposure of an individual or a community to certain EDCs.

One recent breakthrough in microbiome research is the ability to grow desired microbial species based on the need of individuals and their native gut microbiota status. Such
personalized development of microbial therapies will allow considering for sex differences in certain microbial communities and their metabolites. The exploding prebiotic and probiotic markets need to revise their recommendations based on the known sex differences that exist in gut microbiota [72]. Furthermore, the complexity of the microbial ecosystem and the presence of quorum sensing as an additional regulatory mechanism of microbiota homeostasis suggests that combination microbiota therapies that include multiple species may be promising therapeutic targets for metabolic, inflammatory, infectious, and behavioral perturbations.

17.9 CONCLUSIONS

EDCs have wide-ranging effects on targets throughout the body, including the gut microbiome.

The gut microbiome, through metabolism of ingested nutrients, vitamins, hormones, and chemical compounds, can influence the acute and chronic toxicity of EDCs. Sex differences in gut microbiota most likely contribute to differential effects of EDCs between males and females. In addition to the traditional EDC, including plasticizers, xenoestrogens, and metal nanoparticles, ABX also exert endocrine disrupting effects in humans and animals, and thus should be considered as a new class of EDCs. An increase in the use of ABX and a parallel increase in the exposure to other classes of EDCs complicate their effects in exposed individuals and across multiple generations. ABX-treated mice and humans, germ-free mice, and species- and community-specific microbial cultures using in vitro systems will enable the identification of the mechanisms of EDC action on gut microbiome composition and function, microbial metabolites, quorum sensing, and gut morphology. This critical information will provide further insights into the development of less-invasive gut microbial therapeutic targets to mitigate the acute, chronic, and trans-generational effects of EDC.

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