OPINION

Antibiotics in early life and obesity

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Abstract | The intestinal microbiota can influence host metabolism. When given early in life, agents that disrupt microbiota composition, and consequently the metabolic activity of the microbiota, can affect the body mass of the host by either promoting weight gain or stunting growth. These effects are consistent with the role of the microbiota during development. In this Perspective, we posit that microbiota disruptions in early life can have long-lasting effects on body weight in adulthood. Furthermore, we examine the dichotomy between antibiotic-induced repression and promotion of growth and review the experimental and epidemiological evidence that supports these phenotypes. Considering the characteristics of the gut microbiota in early life as a distinct dimension of human growth and development, as well as comprehending the susceptibility of the microbiota to perturbation, will allow for increased understanding of human physiology and could lead to development of interventions to stem current epidemic diseases such as obesity, type 1 diabetes mellitus and type 2 diabetes mellitus.

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Introduction

The human body is host to a vast number of microbes, including bacterial, fungal and protozoan cells, which are present in a far greater number than human cells and collectively constitute our microbiota. Together with their parasitic or perhaps commensal viruses, these microbes carry out a number of functions that are important to human biology, such as aiding development of immunity, protecting against invading pathogens, synthesizing essential vitamins and extracting nutrients from food. The composition of the microbial community is shaped by multiple factors, including the genotype and immunity of the host, as well as environmental influences such as diet, therapeutic agents and direct transmission of microbes through person-to-person contact or transmission through the air, drinking water or food and utensils. These microbial communities are dynamic and the microbiota can be subjected to both minor and major disturbances such as infection, exposure to antibiotics and major dietary shifts.[1,2]

Vertical transmission (which occurs from mother to child during pregnancy, childbirth and in early life), establishment and maturation of the infant intestinal microbiota is a choreographed process that begins in pregnancy (Table 1) and can be perturbed by treatment with antibiotics, changes in diet and interruption of vaginal transmission (for example birth by Caesarean section).[3] Moreover, the microbiota seems to have increased susceptibility to perturbations at some stages of life, particularly during infancy, which is a time before a stable microbial community has developed.[4] Infants acquire much of their founding microbiota at birth (Figure 1), and these microbial populations undergo maturation over the next several years. A microbiota with adult-like complexity is developed by 3 years of age,[5] which corresponds to the age in which infants transition to consumption of a diet similar to that of adult individuals and when major components of acquired (adaptive) immunity are developed.[6] In the first year of life, the microbiota has a beneficial role in shaping healthy host development; however, altered microbiotas at this age have been associated with negative metabolic effects at later stages in life, such as obesity in juvenile individuals.[7] Additionally, breastfeeding has an important role in the selection of the microbiota;[8] milk components can be differentially digested to provide nutrient sources for health-promoting microbes, such as *Bifidobacterium* and *Lactobacillus*. That microbiota composition is both host-specific[9] and conserved across many mammalian species highlights its importance throughout evolution in the past >100 million years. These observations also suggest that optimal development of host–microbiota interactions are those that are orchestrated by shared early-life physiological characteristics and behaviours, including mode of birth, breastfeeding and close interactions with neonatal offspring. Adoption of medical advances such as Caesarean sections, antibiotics and formula feeding might contribute to perturbations in the ancient processes that dictate host–microbiota interactions.

The compromising effects of antibiotics on the important role the microbiota has in mediating colonization resistance (the ability to resist invasion by pathogens) have been reviewed extensively elsewhere.[10] An appreciation of the contribution of antibiotic-induced microbiota disturbances to metabolic changes in the host is emerging. Several studies have established that the intestinal microbiota can modulate host metabolism;[11–13] it is, therefore, plausible that agents that specifically modulate the microbiota, such as antibiotics, can affect body weight. In this Perspectives article, we discuss critical time points in the development of microbiota–host interactions and the sources of early-life microbiota disruption, as well as comment on future research directions.

Antibacterial exposure

The patterns of microbial colonization in early life can be disrupted by altering the composition of founding microbial populations and/or through exposures to antibiotics during infancy. Maternal antibiotic exposure is a relevant consideration, as infants acquire at least a part of their early life microbiota from their mothers. Antibiotic exposure immediately prepartum, as occurs in >30% of US women to prevent Group B *Streptococcus* infection,[14] could have a direct effect on the vertical transmission of microbiota. However, the effects of antibiotic exposure in early pregnancy, or even before pregnancy, on maternal transmission of microbiota to infants have not been

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established. Additionally, the maternal microbiota composition shifts between the first and third trimester of pregnancy and these changes are conserved across species,[3] which suggests that they confer evolutionary advantages for fecundity and infant survival; these changes might also be subjected to disruption with antibiotics. Our group has postulated that maternal exposure to antibiotics could affect intergenerational transmission of microbiota.[15]

A large population-based Danish study reported that 78% of mothers received an antibiotic during a 4 year period that spanned before, during and after pregnancy; of these women, 51% had received three or more courses of antibiotics during the study period.[16] Interestingly, the likelihood of a child developing asthma was increased when the number of courses of antibiotics taken by their mother increased, with an overall increased risk of 30% in the children of mothers who used antibiotics. One interpretation of these findings is that maternal antibiotic use has a direct effect on the microbiota and physiology of infants even after birth. An examination of another cohort of Danish women showed that more than 40% received an antimicrobial agent at least once during pregnancy.[17] In addition to disrupting the transmission of the microbiota from mother to child, prenatal antibiotic exposure has been shown to have effects on the birth weight of neonates and is associated with increased risk of obesity and related metabolic sequelae later in life.[18,19]

In the USA, infant exposure to prescribed antibiotics is substantial. Analysis of antibiotic prescription rates in 2010 from a database containing information on >70% of prescriptions in the USA[20] demonstrated widespread use of antibiotics, especially during infancy and childhood. Our extrapolation of the data suggests that, on average, a child in the USA has received nearly three antibiotic courses (largely to treat acute infections of the ears and upper respiratory tract) by the age of 2 years, about 10 courses by the age of 10 years and ~17 courses by 20 years of age. Although these rates are astonishingly high, they are consistent with those from prior national surveys.[21,22] By contrast, in Sweden, antibiotic use from infancy through adulthood is ~40% of that in the USA.[23] This dichotomy suggests that much of the prescribed antibiotic use in US children is unnecessary—a fact that is widely acknowledged by professional bodies, including the American Academy of Pediatrics[24] and the Centers for Disease Control and Prevention.[25] However, it should also be noted that rates of paediatric antibiotic use in the USA did decrease from 2000 to 2010.[26] Furthermore, the data from the 2010 US survey also indicated that regional differences exist in antibiotic prescription rates, with higher rates in the south than in the west of the country.[20,27] Even within a single region, considerable variation of prescribing among doctors was observed, as indicated by a large survey of practices associated with a major academic medical centre.[28]

US infants could potentially have substantial exposure to antibacterial agents from other sources such as the food supply chain or drinking water.[29–31] Given that antibiotics are widely used for promotion of growth in livestock, meats and milk from these animals might be contaminated with trace residues of these agents.[32] However, stringent regulations are in place in the USA that aim to limit these types of exposures.[29,33] Concerns that use of antibiotics in livestock promotes the emergence of antibiotic-resistant bacterial strains that adversely affect human health are long-standing.[34] Moreover, some studies have drawn attention to the question of whether consumption of contaminated products could have direct metabolic effects on human health.[29,35,36]

Table 1 | Choreography of microbiota transmission, establishment and maturation

Stage	Key microbiota components	Sources of perturbation*
Pregnancy	Increased levels of lactic acid bacteria in the third trimester[3]	Maternal antibiotics[85]
Birth	Transmission of vaginal microbiota, *Lactobacillus*, *Bifidobacterium* and *Streptococcus spp.*, followed by an increase in *Enterobacteriaceae* populations[86]	Caesarean section[87] Antibiotic treatment at delivery[88]
Nursing	Predominance of *Bifidobacterium* and *Lactobacillus* spp.[8]	Formula feeding[8,89] Antibiotic treatment[4,86]
Solid foods	Increase in obligate anaerobic populations (for example, *Clostridium* and *Bacteroides*)[89]	Antibiotic treatment[4] Sanitizers

*The sources of perturbation can be cumulative, with additive or progressive disruption from multiple insults (such as antibiotics, altered modes of delivery or lack of breastfeeding).

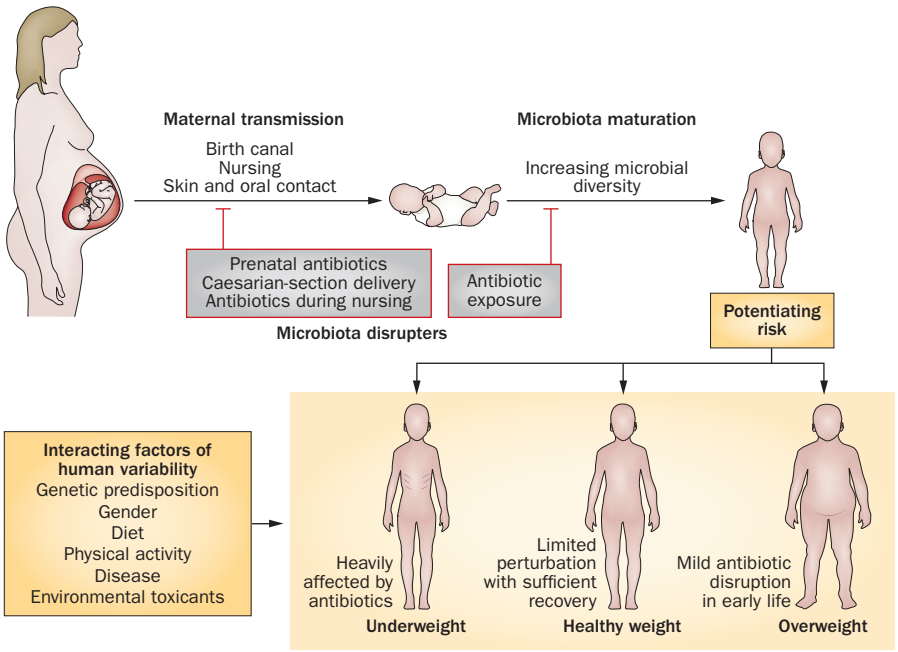

Figure 1 | A model of microbiota transmission, maturation and perturbation in early life and possible effects on weight. Infants receive much of their colonizing microbiota at birth, during nursing (via breast milk) and through maternal interactions (skin contact). Microbial communities might be affected by maternal antibiotic use or by circumventing normal colonization routes. In infancy, the microbiota is particularly vulnerable to antibiotic disruption, and having an altered microbiota can affect growth and development later in life, with consequences such as excessive weight gain or stunted development. Other factors can affect metabolic development, including genetic predisposition, sex, diet, physical activity, disease and environmental toxicants.

The overall risk of exposure to antibiotics via contaminated meat and dairy products might be related to variations in global regulatory and testing policies.[32] Increased levels of exposure might result from ingesting meat, dairy and egg products imported from countries with less stringent regulations than the USA. Use of subtherapeutic doses of antibiotics is permitted for meat production in the USA, although the FDA regulates the type of antibiotics used and, in theory, the allowable levels of residues in meat.[37] In December 2013, the FDA revised this policy and requested voluntary withdrawal of use of antibiotics for growth promotion;[38] if the policy proves to be effective, incidences of unwanted antibiotic exposure could be curtailed.[39] In 2011, of the 5,006 meat samples tested by the FDA from a variety of animals, 47 had detectable levels of antibiotic residues and eight samples had levels above the allowed limit.[33] Increased levels of antibiotic contamination were also reported in surveys conducted before 2011. For example, rapid screening methods for detection of sulphonamide that were introduced in 1984 enabled farmers to determine whether levels of antibiotics in calves exceeded the allowable limit (thus needing an increased washout period) or were within acceptable limits; implementation of this measure decreased the proportion of samples above the violation limit from 5% to 2% in just 18 months.[40] Meat from farmed fish also frequently has detectable levels of antibiotic residues, which raises concern that practices in aquaculture might introduce antibiotics into the human food supply.[41]

In some US communities, having municipal water intake downstream of farm effluents might lead to potable water having trace levels of antibiotics.[30] These exposures could affect the health of infants either directly or through consuming their mothers' milk. Extensive washing and bathing of infants with antibacterial soaps and the ingestion of antibacterial preservatives in food might also contribute to altering the early-life microbiome, possibly in synergy with the disruptive effects associated with Caesarean-section births and prescribed antibiotics. Although implementation of hygiene practices that prevent serious bacterial infectious disease and avoid the need for antibiotics might be beneficial, where to set the limit is unclear. The extent to which these nonmedical exposures influence development of the early-life microbiome, as well as their consequences on health later in life, are currently unknown, but are important topics for future research.

Antibiotics and metabolism

Evidence from farm animals

Approximately 70 years ago, veterinary scientists showed that adding low (subtherapeutic) doses of antibiotics to the food or water of pigs resulted in promotion of growth.[42] This effect has subsequently been shown in other common types of mammalian livestock (cows and sheep) and in poultry.[43] A wide variety of antimicrobial agents has been demonstrated to have these effects regardless of class of drug (antibiotic, ionophore or antiseptic), chemical structure, mode of action and spectrum of activity.[44–46] Importantly, when animals are exposed to antibiotics early in life, the effects on both growth promotion and feed efficiency (the ability to convert food calories into body mass) are greater than if the exposure occurs later in life.[44,46] The effects associated with age are consistent with the concept of a critical developmental period for shaping host metabolism, with early life being more vulnerable to change than late life. Antibiotic-mediated promotion of growth is widely practiced by farmers because it is very effective. Studies in germ-free chickens have shown that antibiotics alone have no growth-promoting effects,[47] which provides evidence against a direct effect of antibiotics on host tissues but suggests that the effects are driven by changes in the microbiota of treated animals.

Together, the findings from observations and experiments in livestock and poultry indicate that early life is a critical time for metabolic development of the host, that the microbiome has a role in this process and that antibiotic exposure at this time affects the course of growth and development.

Evidence from animal models

Several studies have shown that the intestinal microbiota influences host metabolism,[11,12,48] which supports the concept that treatment with agents that modulate commensal microbial populations can affect the weight of the host (Table 2). Experiments in animal models[49,50] have provided direct evidence of a link between treatment with low doses of antibiotics and growth promotion, a relationship that had been suggested by the early studies in farm animals.[43] Studies in mice using multiple types of antibiotics have further confirmed this association,[49] as well as identifying early life as the key period for microbe-mediated programming of host metabolism.[50] One hypothesis that emerged from the farm studies was that use of antibiotics leads to growth promotion by reducing infection; however, the antibiotic-mediated effects on metabolism were also observed in mice that were reared in specific pathogen-free conditions.[49,50] Furthermore, given that the low doses of antibiotics administered in the farm setting are well below therapeutic levels, it is unlikely that these treatments would be sufficient for clearance of pathogens.

Administration of low doses of penicillin or oxytetracycline has been shown to lead to weight gain in mice, but high doses lead to weight loss.[51] Treating mice with subtherapeutic doses of penicillin, vancomycin and chlortetracycline led to increased fat mass and increased levels of short-chain fatty acids in these animals, which suggests that the altered microbiota had an enhanced metabolism that could drive induction of downstream genes involved in hepatic lipogenesis.[49] Different antibiotic treatment regimens target specific populations of bacteria. Penicillin (a β-lactam antibiotic) and vancomycin (a glycopeptide) both inhibit cell wall synthesis in Gram-positive organisms; chlortetracycline, which inhibits protein synthesis, has very broad-spectrum activity.[26] Increases in fat mass were seen in all mice treated with antibiotics, regardless of the antibiotic class,[49] which is consistent with findings from studies conducted in farm animals.[43] Additionally, in the agricultural setting, the efficacy of a wide range of antibiotics, including diterpenes, lincosaminides, macrolides, oligosaccharides, peptides, streptogramins, phosphoglycolipids, polyethers, quinoxalines and sulphonamides, has been observed.[44]

That treatment with antibiotics from a wide array of classes results in increased fat mass suggests that generalized disruption of the gut microbiota can alter host metabolism. Furthermore, mice that received low-dose penicillin (LDP) treatments at birth had greater increases in total body weight than their counterparts that were exposed to LDP at weaning and to control mice,[50] which indicates that early life is a metabolically vulnerable stage. Challenging mice that received LDP at birth with a high-fat diet accentuated the antibiotic-mediated metabolic effects, demonstrating synergy between the effects from early-life microbiota disruption and dietary excess. Importantly, these metabolic effects lasted into adulthood even after the treatment with antibiotics was terminated. Mice that received LDP for only 4 weeks after birth developed obesity and significantly increased fat mass starting at 20 weeks.[50] This effect was not a result of a sustained dysbiosis: 4 weeks after penicillin treatment was stopped, the microbiota had

Table 2 | Experimental evidence of the effect of antibiotics or disrupted microbiota on host weight

Study	Strain or species	Treatment (dose, timing)	Diet	Effect on weight	Effect on microbiota
Bäckhed *et al.* (2007)[68]	C57BL6J mice	Germ-free (NA, lifelong)	Western	Less weight gain than similarly fed conventionalized mice	Absent
Fleissner *et al.* (2010)[70]	CH3 mice	Germ-free (NA, lifelong)	Chow HFD Western	No change Increased weight gain Reduced fat gain	Absent
Coates *et al.* (1963)[47]	Germ-free chickens	Penicillin (45.5 mg/kg diet, from birth)	Chow	No effect on weight	Absent
	Chickens	Penicillin (45.5 mg/kg diet, from birth)	Chow	Weight gain	Not done
Dubos *et al.* (1963)[51,88]	NCS mice	Penicillin or oxytetracycline (0.3 g/l water, starting at age 30–33 days for 1 week)	15% gluten*	Weight loss	Oxytetracycline: loss of *Lactobacilli* and Gram-negative *Bacilli*, increase in *Enterococci*
		Oxytetracycline (0.3 g/l, starting at age 30–33 days for 1 week)	Pellets‡	Weight gain	
		Penicillin or oxytetracycline (0.3 g/l, starting at age 30–33 days for 1 week)	15% casein§	Weight loss	
		Penicillin (1 g/l, starting at age 30–33 days for 1 week)	15% casein§	Weight loss	Loss of *Lactobacilli* and *Enterococci*
		Penicillin (0.1 g/l, starting at age 30–33 days for 1 week)	15% casein§	No change	Reduced number of *Lactobacilli*
	Ha/ICR mice	Penicillin or oxytetracycline (0.3 g/l, starting at age 30–33 days for 1 week)	Pellets‡ 15% casein§	Weight gain	Oxytetracycline: loss of *Lactobacilli* and Gram-negative *Bacilli*, increase in *Enterococci* Penicillin 0.1 g: reduced number of *Lactobacilli* Penicillin 1 g: loss of *Lactobacilli* and *Enterococci*
Membrez *et al.* (2008)[65]	*ob/ob* mice	Norofloxacin and ampicillin (1 g/l drinking water, starting at age 8–10 weeks for 2 weeks)	Chow	Reduced fat mass	Near elimination of aerobic bacteria, 3-log reduction in the number of anaerobic bacteria
Cani *et al.* (2008)[52]	C57BL6 mice	Ampicillin and neomycin (1 g/l [ampicillin], 0.5 g/l [neomycin] starting at age 12 weeks for 4 weeks)	HFD	Reduced weight	Increased number of *Bifidobacterium*, reduced numbers of *Lactobacillus* and *Bacteroides* (*Prevotella*)
	ob/ob mice	Ampicillin and neomycin (1 g/l [ampicillin], 0.5 g/l [neomycin] starting at age 6 weeks for 4 weeks)	Chow	Reduced fat mass	Reduced *Bifidobacterium*, Lactobacillus and *Bacteroides* (*Prevotella*)
	ob/ob CD14KO mice	None (NA, lifelong)	Chow	Reduced fat mass	Not done
Carvalho *et al.* (2012)[64]	Swiss mice	Ampicillin, neomycin and metronidazole (1 g/l, at age 8 weeks)	HFD	Reduced weight gain	Considerable depletion of *Bacteroidetes* and *Firmicutes*, multilog-fold reduction in anaerobic and aerobic bacterial counts
Cox *et al.* (2014)[50]	C57BL6 mice	Penicillin (1 mg/kg body weight [7 mg/l drinking water], at birth or age 4 weeks, then lasting through life)	Chow	Greater increase in weight when administered at birth than at 4 weeks, increased effect in male mice	No reduction in total microbial populations
	C57BL6 mice	Penicillin (1 mg/ kg body weight [7 mg/l drinking water], lifelong)	HFD at 17 weeks	Promotion of diet-induced obesity and related metabolic effects	Consistently reduced number of *Lactobacillus*, *Allobaculum*, Rikenellaceae and *Candidatus arthromitus* (SFB)
	C57BL6 mice	Penicillin (1 mg/ kg body weight [7 mg/l drinking water] During the first 4 weeks, the first 8 weeks or lifelong	HFD at 6 weeks	Increased total, lean and fat mass in all groups (greater effect in female mice than male mice)	Consistently reduced number of *Lactobacillus*, *Allobaculum*, Rikenellaceae and *Candidatus arthromitus* (SFB)
	Germ-free Swiss–Webster mice	Microbiota from antibiotic-treated mice (NA, at age 3 weeks)	HFD	Increased fat and total mass in recipients of microbiota from antibiotic-treated mice	Reduced number of *Lactobacillus*, *Allobaculum* and Rikenellaceae

Table 2 (Cont.) | Experimental evidence of the effect of antibiotics or disrupted microbiota on host weight

Study	Strain or species	Treatment (dose, timing)	Diet	Effect on weight	Effect on microbiota
Cho *et al*. (2012)[49]	C57BL6 mice	Penicillin or vancomycin Chlortetracycline Penicillin and vancomycin (1 mg/kg body weight, from age 3 weeks through life)	Chow	Increased fat mass in all antibiotic groups	Increase in *Lachnospiraceae*
Murphy *et al*. (2013)[66]	C57BL6 mice	Vancomycin (2 mg/day, at age 12–20 weeks)	HFD	Weight loss	Reduced number of *Clostridium* and *Bacteroides*, rise in number of *Enterobacteriaceae*
Morel *et al*. (2013)[67]	Sprague–Dawley rats	Amoxicillin (150 mg/kg body weight, at age 5–15 days)	Chow	No change in weight	Reduced *Bacteroides*, *Lactobacillus* and *Clostridium leptum* cluster at day 21 of life

*Gluten as a sole protein source (deficient in lysine and threonine). ‡Complex mouse diet from Dietrich and Gambrill, Frederick, MD, USA. §Casein as a sole protein source. Abbreviations: HFD, high-fat diet (45% calories from fat); NA, not available; SFB, segmented filamentous bacteria.

recovered; however, the mice still developed adult-onset obesity. These findings demonstrate that even transient perturbations in the early-life period, during which the microbiota contributes to normal host development, can have long-term effects. Additionally, the altered microbiota itself was capable of producing the obesogenic effect; young (3-week old) germ-free mice that were colonized with microbiota from LDP-treated mice gained more weight and fat mass than mice colonized with microbiota from control animals. Throughout these experiments, consistent reductions in the population size of specific microbiota, such as *Lactobacillus*, *Allobaculum*, Rikenellaceae and *Candidatus arthromitus* (also known as segmented filamentous bacteria, SFB) were observed, which suggests that bacteria of these taxa might have protective roles in shaping adult metabolism.[50]

One specific interaction that might be affected by changes in the microbiota is the guidance of the development of intestinal immunity by the microbiota; reductions in intestinal defence can lead to metabolic aberrations.[52–55] Treatment of mice in early life with LDP or colonization with microbiota from LDP-treated mice resulted in decreased expression of genes in the ileum that are involved in regulating multiple functions associated with development of innate and adaptive immunity, including antigen presentation and generation of type 17 T helper (T_H17) cell responses and antimicrobial peptides. One population of bacteria that was reduced in these mice as a result of LDP treatment was that of SFB, which stimulates T_H17 responses and antimicrobial peptide secretion.[56] Furthermore, experiments performed in mice[57] and limited evidence from studies in humans[58] have shown that high levels of SFB are present in infancy, that that these levels are reduced in adulthood and can be completely lost after penicillin exposure.[59]

We believe that other organisms have important functions in the ileum; however, SFB is considered a model organism with levels that peak in infancy and that can guide developmental outcomes. Loss of this population in early life has been shown to have adverse consequences, such as increased vulnerability to infection by the intestinal pathogen *Citrobacter rodentium*.[56] Other populations within the microbiota can drive development of specific immune responses. For example, presence of *Clostridia* species from clusters IV and XIVa leads to increases in the numbers of regulatory T cells (which secrete the anti-inflammatory cytokine IL-10) in the lamina propria.[60] *Bacteroides fragilis* strains that contain polysaccharide A (PSA) also induce mucosal IL-10 secretion, which highlights the importance of specific cell wall components in this process.[60] Presence of both of these organisms in the microbiota can also modulate systemic immunity; colonization with a cocktail of *Clostridia* species from clusters IV and XIVa resulted in decreased circulating levels of IgE after challenge with a presensitized antigen,[61] and colonization with a PSA-positive *B. fragilis* strain increased numbers of circulating type 1 T helper cells.[62] Further studies are warranted to investigate the individual mechanisms by which key early-life members of the microbiota shape host immunity.

Many studies have shown that early life is a critical time for host metabolic development; however, research has also shown that exposure to high doses of antibiotics early in life can stunt growth and lead to underdevelopment[51] or ameliorate metabolic outcomes when animals are challenged with a high-fat diet.[52,63,64] In rodent models of obesity, high doses of antibiotics that resulted in log-scale reductions in numbers of anaerobic and aerobic bacteria, considerably decreased body weight and/or fat mass and improved markers of insulin sensitivity.[52,64,65] It seems paradoxical that some exposures to antibiotics can lead to weight loss,[51,52,66] yet others result in weight gain,[49–51] and some exposures have no direct effect on weight.[67] Antibiotic exposures that either increase or decrease body weight might differentially alter feeding behaviours[67] or metabolic signalling, which are factors that can be modified during the early-life developmental period.[63] These divergent phenotypic outcomes might explain why the effects of antibiotic treatments on weight remained largely unnoticed in clinical practice, whereas the growth promotion induced by subtherapeutic doses of antibiotics was recognized by farmers decades ago.[43]

The variations in metabolic outcomes that are associated with antibiotic exposure seem to be largely dependent on the dose of antibiotics, timing of administration of these drugs, mouse strain and diet; the opposing effects on body weight might depend on the overall magnitude of disruption to the microbiota (Figure 2). The intestinal microbiota contribute to host calories by extracting energy from the diet,[12] and loss of microbiota (as in the case of germ-free mice) results in abnormal host metabolism, physiology and immunity. Germ-free C57BL6 mice weigh less than their conventional counterparts despite similar food intakes. These mice also resist weight gain when exposed to either a Western[68] or a high-fat diet,[69] as a result of decreased energy extraction and altered expression of genes related to energy homeostasis (such as *Fiaf*, the fasting-induced adipose factor).[50] However, this effect is not universal and might be influenced by factors such as host genetics and diet composition,

as germ-free CH3 mice have increased weight gain when exposed to a high-fat diet and reduced fat mass when fed a Western diet.[70] Treatments with antibiotic regimens that produce marked population reductions might create conditions that parallel the germ-free state or could lead to undernutrition.[51,52,64,65] These effects were magnified when mice were fed a diet that was deficient in specific nutrients, such as essential amino acids, and the weight-reduction effect was lost when mice were fed a complete diet.[51]

Epidemiologic evidence

A number of epidemiological studies have tested the hypothesis that exposure to antibiotics in early life is associated with increased risk of excess adiposity. In a study of over 28,000 mother–child pairs from the Danish National Birth Cohort,[71] antibiotic exposure in children during the first 6 months was associated with an increased risk of being overweight at 7 years of age; the effect was stronger in boys than in girls. These findings were supported by those from the Avon Longitudinal Study of Parents and Children (ALSPAC), which included over 10,000 children.[72] In the ALSPAC birth cohort, when all known confounders were controlled for, antibiotic use in the first 6 months of life was associated with increased BMI at 10, 20, and 38 months of age, which was consistent with the strong effects of exposures to antibiotics in early life seen in farm animals. The Danish and the ALSPAC studies also determined that maternal BMI was a contributing factor for the development of obesity following exposure to antibiotics in early life, with increased effects seen in children with mothers of normal weight compared with children from mothers who were overweight. In studies of Canadian infants, antibiotics administered in the first year of life increased the likelihood of a child being overweight at 9 years and 12 years of age, as well as having elevated central adiposity (a marker of the metabolic syndrome).[73] These effects were observed after adjustment for other factors known to influence body weight, such as diet, physical activity, having siblings and maternal smoking during pregnancy. Strong sexual dimorphism was apparent, with the effect being almost entirely seen in male children. A longitudinal study in the USA also showed an association between early-life antibiotic use and childhood obesity, which was increased with early exposure and multiple treatment courses.[74] Interestingly, these effects were considerably associated with use of broad-spectrum antibiotics, but not with use of narrow-spectrum antibiotics. Finally, in a global cross-sectional study, antibiotic treatments in the first year of life modulated body weight in children, and changes in both directions (increases or decreases) were dependent on both site and country, and an overall association with increased risk of being overweight at 5–8 years of age was observed in boys.[75] In the same study, the weight gain or weight reduction observed in female children was dependent on the study site; however, overall, no statistically significant effects were observed in this group of participants. Together these epidemiological studies provide evidence that exposures to antibiotics very early (that is, in the first year of life) could affect the risk of excess adiposity later in life, which implies that these effects occur during a critical developmental period. The sex-specificity of these effects, if sustained, remains unexplained.

Colonization of an infant with their microbiota relies on vertical transmission from the mother at the time of delivery; thus, maternal exposure to antibiotics or an altered delivery route could also affect the proper establishment of the microbiota and, consequently, have effects on weight gain. A study of 436 mother–child pairs found an average of 84% (range of 33–154%) increased risk of obesity at 7 years of age if the mother received antibiotics in the second or third trimesters of pregnancy.[60] Increased risk of having obesity or being overweight has been associated with delivery by Caesarean section in several independent studies.[60,76,77] These studies provide evidence that transmission of maternal microbiota is likely to be a critical factor that shapes metabolic development in children.

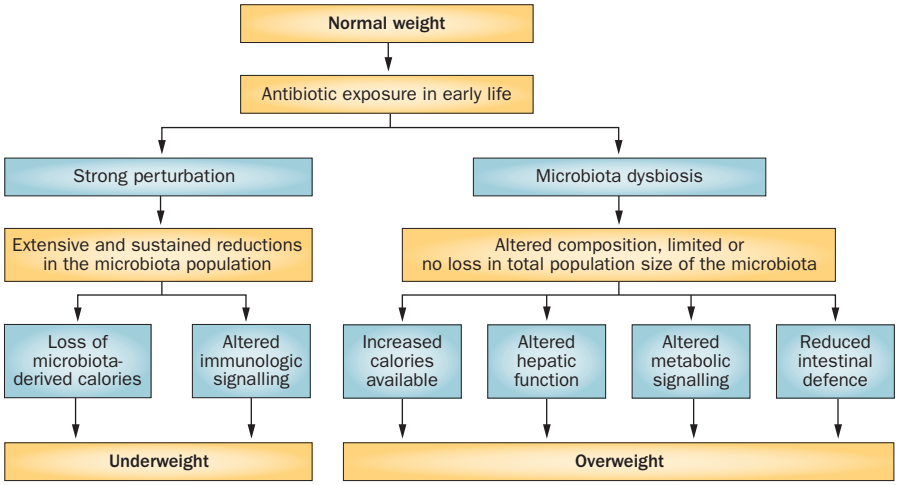

Figure 2 | Proposed pathways of antibiotic-mediated weight modulation. Exposure to high-dose antibiotics can cause extensive reductions in microbiota populations early in life and can lead to underdevelopment and stunted growth.[51] These perturbations might alter immunological signalling or decrease production of microbiota-derived calories and nutrients leading to weight loss. Antibiotic treatments in early life that alter microbiota composition but have limited effects on the overall microbial population size can result in weight gain (by reducing populations of metabolically protective bacteria, increasing production of microbiota-derived calories and altering hepatic metabolic signalling and/or intestinal defences). Disturbances to the microbiota would probably need to exceed a specific threshold to yield clinical phenotypes.

Therapeutic intervention

Administration of probiotics to mothers in the last month of pregnancy in humans[78] or in early life in animal models[79] results in either increased or decreased growth and weight of offspring; whether one or the other of these outcomes occurs possibly depends on the strain of probiotic and host species. Nevertheless, these studies support the idea that specific populations within the microbiota can influence weight. Additional studies are needed to determine whether probiotics can influence host growth and development following antibiotic therapy.

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".[80] These agents can be purchased without a prescription in many countries and can be used without the requirement of a specific health need. Following antibiotic treatment in the clinical setting, we believe the terminology 'targeted restoration bacteriotherapy' helps to delineate future strategies for improving health outcomes that result from disruption of microbiota in early life. Currently available probiotics are

limited to a relatively small number of phylogenetic lineages compared with the highly diverse microbiota present in the developing infant and adult individuals; thus, the need to identify additional bacterial therapeutic targets remains unmet. Moreover, the idea of restoration following treatment with antibiotics (to replace what is lost) should be a guiding principle of probiotic use; administration of bacteria might not be required in an individual who does not have an obviously antibiotic-affected microbiota. Lastly, the term bacteriotherapy[81] highlights the aim of counteracting a disease, and in the case of early-life antibiotic exposure, would be considered a preventive measure. We believe that differentiation of these terms will direct the careful study of clinical outcomes in the future, but given that the field is nascent, much remains to be learned.

Conclusions

The assembly of the intestinal microbiota is intimately associated with normal human growth and development.[82] In healthy individuals, the gut microbiome is resilient and able to form stable communities that maintain particular compositional and functional characteristics across generations of individuals. Across the human population, the gut microbiota forms a community structure that is unique compared with those of oral or skin microbiota, which indicates that there are active forces (including pH, immunity, specific nutrients, limited oxygen, a high flow rate and microbe–microbe interactions) that select for specific conditions, which drive resilience and recovery following environmental perturbations. Despite homeostatic mechanisms, antibiotic treatments can lead to long-term alterations in microbiota composition[83] that result in changes to host metabolic functions,[63] particularly during development.[50]

Our work has demonstrated lasting metabolic consequences from transient disruption to the microbiota in early-life despite eventual recovery.[50] With the aim of reversing some of the metabolic consequences resulting from treatment with antibiotics, strategies to restore the microbiota might need to account for the timing of interventions. If the recovery to equilibrium could be accelerated, is it possible to prevent later metabolic sequelae? Importantly, mice treated with penicillin for the first 4 weeks of life showed delayed, but eventual, recovery of microbiota populations; nevertheless, they still developed increased fat mass weeks after microbiota recovery.[50] The sensitivity of the metabolic development of the host during this period might indicate that restoration of the microbiota immediately following treatment with perturbing antibiotic therapies could be an important preventive measure.

Ultimately, antibiotics are important and potentially life-saving drugs that have considerably reduced the rates of human mortality and morbidity. Although these agents were thought to have minimal long-term metabolic adverse effects, we are now gaining clear insights to how these microbiota-modulating agents might contribute to obesity. By understanding the metabolic costs associated with these treatments, we can factor them into the equation of clinical guidelines and decisions to continue to support the prudent use of antibiotics.

Many unanswered questions warrant research to eventually permit the translation of the findings summarized here to the general human population. Additional longitudinal studies in humans that specify antibiotic exposures (such as dose, class and timing) better than the studies performed to date could help assess the risks associated with use of these drugs. We must increase the understanding of the extent of perturbation required to elicit metabolic effects, as compounded disruptions can have unexpected or magnified effects.[84] Finally, little is known about the potential to reverse the metabolic effects of microbiota disruption in early life. Targeted restoration bacteriotherapy administered at the right time could be beneficial.

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