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Antibiotics, gut microbiome, and obesity

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Abstract

Antibiotics have been hailed by many as ‘miracle drugs’ that have been effectively treating infectious diseases for over a century, leading to a marked reduction in morbidity and mortality. However, with the increasing use of antibiotics, we are now faced not only with the increasing threat of antibiotic resistance, but also with a rising concern about potential long-term effects of antibiotics on human health, including the development of obesity. The obesity pandemic continues to increase, a problem that affects both adults and children alike. Disruptions to the gut microbiome have been linked to a multitude of adverse conditions, including obesity, type 2 diabetes, inflammatory bowel diseases, anxiety, autism, allergies, and autoimmune diseases. This review focuses on the association between antibiotics and obesity, and the role of the gut microbiome. There is strong evidence supporting the role of antibiotics in the development of obesity in well-controlled animal models. However, evidence for this link in humans is still inconclusive, and we need further well-designed clinical trials to clarify this association.

Introduction

Antibiotics have been hailed by many as the ‘miracle drugs’ that have revolutionised the medical field since their introduction over a century ago¹. By effectively treating infectious bacterial diseases, they have led to a marked reduction in morbidity and mortality¹. However, with the increase use of antibiotics, we are now not only faced with the increasing threat of antibiotic resistance, but with a rising concern that there are other potential long-term effects of antibiotics on human health, including the development of obesity². This review will focus on the association between antibiotics and obesity, and the role of the gut microbiome. The gut microbiome has been recognised to be linked to a multitude of diseases, including obesity, type 2 diabetes, inflammatory bowel diseases, anxiety, autism, allergies and autoimmune diseases³⁻⁷.

How big is the obesity problem?

According to the World Health Organization, 1.9 billion adults were overweight and 600 million were obese in 2014⁸. Children have not been spared, whereby a staggering 41 million children aged <5 years were estimated to be overweight or obese in 2014⁸. A comprehensive global study by the NCD Risk Factor Collaboration showed that the incidence of obesity in men increased 3.4-fold between 1975 and 2014 (from 3.2% to 10.8%), with a 2.3-fold increase among women (6.4% to 14.9%)⁹. Similar trends were noted in children, whereby the worldwide prevalence of childhood overweight and obesity increased 1.6 fold between 1990 and 2010 (4.2% to 6.7%). This trend is expected to increase more than two fold to 9.1% or 60 million children by 2020¹⁰.

Is the gut microbiome associated with obesity?

The human gut is colonised by a vast array of microorganisms, including bacteria, viruses, archaea, and protozoans^{11,12}, which are collectively known as the gut microbiome¹³. The number of bacteria in particular is so vast that it approximates the number of human cells¹². Colonization of the gut begins before birth and is influenced by a variety of dietary and environmental factors^{4,14,15}. There is increasing evidence that the gut microbiome has a range of effects on the host's physiological processes and behaviour¹⁶⁻¹⁹.

Dysbiosis (imbalance in gut microbiota) has been linked to the development of obesity by multiple mechanisms, via either direct effects on the gut or indirect regulation of distal organs^{17,20} (Figure 1). These bacteria are able to break down indigestible polysaccharides (fibre) to short-chain fatty acids (SCFAs), which provide 80-200 kcal/day of energy to normal adults¹⁶. Dysbiosis (for example a 20% increase in Firmicutes and a corresponding 20% decrease in Bacteroidetes) can result in an additional 150 kcal of energy harvested per day^{11,19}. SCFAs also modulate the secretion of gut hormones (glucagon-like peptide (GLP-1) and peptide YY), both of which directly influence satiety¹⁸ (Figure 1).

In addition, the gut bacteria have an effect on bile acid metabolism, leading to a reduction in the number of bacteria considered to be protective against obesity²¹ (Figure 1). Bile acids acting as ligands of the nuclear farnesoid X receptor (FXR) and G-protein coupled bile acid receptor 1 (TGR5) are postulated to be involved in the regulation of gut hormone secretion, as well as in glucose and lipid metabolism²²⁻²⁵ (Figure 1).

The gut microbiome also has the ability to disrupt the gut mucosal barrier leading to increased exposure of the host's immune system to bacterial products^{16,18} (Figure 1). Increases in certain bacteria, and consequently on the concentration of their membrane lipopolysaccharides (LPS), have been proposed to lead to a condition known as metabolic endotoxaemia^{18,26}. Endotoxaemia along with increased gut permeability is associated with greater inflammation, which in turn leads to weight gain, fasting hyperglycaemia and hyperinsulinaemia^{16,18,26} (Figure 1). Dysbiosis also reduces the production of angiopoietin-like protein 4 (ANGPTL4) that inhibits lipoprotein lipase, leading to excess deposition of triglycerides in the adipose tissue, liver, pancreas, and heart²⁷ (Figure 1). Other metabolic effects of the gut bacteria are caused by changes in behaviour, including appetite modulation, food intake¹⁸, and energy expenditure¹¹ (Figure 1).

The published evidence linking gut microbiome to obesity is summarized in Table 1. Most of what is known about this relationship comes from intervention studies in mice²⁸⁻³¹. One study by Backhed et al. in 2004 noted that conventionally raised mice had significantly more body fat compared with mice raised under germ-free conditions²⁸. Introduction of bacteria from the caecum of conventionally raised

mice to the germ-free ones produced a 60% increase in body fat within 2 weeks despite reduced food intake²⁸ (Table 1). They also demonstrated that upon colonisation of germ-free mice with the gut bacteria from conventionally raised mice, fasting leptin, glucose, and insulin levels were increased leading to increasing body fat content. Further, the ANGPTL4 levels were suppressed in the gut epithelium leading to elevated lipoprotein lipase activity, resulting in increasing triglyceride storage in adipocytes²⁸ (Table 1). In 2006, Turnbaugh et al. showed that transferring gut bacteria from obese mice to germ-free ones led to increased fat mass in the recipients³¹ (Table 1). Turnbaugh et al. further demonstrated that transplanting obesogenic gut microbiome to germ-free mice resulted in nearly a 3-fold increase in fat mass compared to mice who received gut microbiome from lean donors³² (Table 1).

More recently, Ridaura et al. evaluated the effects of an obesogenic gut microbiome transfer from human donors to mouse recipients³³ (Table 1). In contrast to earlier studies in mouse donors and recipients, they demonstrated that introduction of faecal microbiota from an overweight adult twin to normal-weight germ-free mice resulted in a rapid weight gain in the mice. However, mice treated with faecal microbiota from the lean twin maintained their normal weight³³ (Table 1). Further, compelling evidence of the effect of the gut microbiome on obesity comes from a study on gut microbiome transfer from human donors post-bariatric surgery to mouse recipients, which promoted a reduction in fat deposition in recipient mice³⁴ (Table 1). Very recently, Liu et al. showed that obese humans have fewer *Bacteroides thetaiotaomicron* (glutamate-fermenting bacteria) in their gut, with an associated increase in serum glutamate concentrations³⁵ (Table 1). Transfer of *B. thetaiotaomicron* led to a reduction in serum glutamate levels, significantly reducing total fat mass and increasing lean body mass in mice on normal diet, and preventing further weight gain and adiposity in mice on a high-fat diet³⁵ (Table 1). Bariatric surgery was also reported to alter the gut microbiome by increasing *B. thetaiotaomicron* numbers³⁵. As a result, there was an associated reduction in serum glutamate levels and improvement in metabolic parameters including insulin resistance³⁵ (Table 1).

Studies on the composition of the gut microbiome have shown that there is a difference between the bacterial taxa in lean and obese individuals³⁶⁻⁴⁰. There is however, a paucity of human intervention studies to determine whether the gut microbiome does in fact influence body weight or whether previous observations were merely associations without a causal link. Dietary-intervention studies reveal that restrictive diets seem to result in a reduction of gut microbiota diversity and butyrate producers (Firmicutes, *Lactobacillus* spp., and *Bifidobacterium* spp.), which is associated with macronutrient deficiency rather than weight loss^{41,42}. Data from bariatric surgery indicate that a similar reduction in butyrate producers was also observed, depending on the surgical technique used⁴³. This may result in reduction of energy harvested from food and SCFAs leading to reduction in gluconeogenesis and lipogenesis⁴³. Studies on the use of probiotics as gut microbiota interventions

have yielded conflicting results, but generally showed a reduction in body fat⁴⁴⁻⁴⁸ (Table 1). Further, studies on prebiotics indicate that these compounds are able to promote changes in both composition and function of the gut microbiota⁴⁹⁻⁵¹. They increase the number of *Bifidobacterium* spp. and other butyrate producers, leading to improvements in metabolic outcomes and the gut barrier against pathogens⁵². However, prebiotic intervention is affected by the individual's gut microbiota composition and fibre intake⁵³. Nicolucci et al. recently demonstrated in a randomised placebo-controlled trial that the prebiotic oligofructose-enriched inulin altered the gut microbiota in children (mainly increasing *Bifidobacterium* spp. and decreasing *Bacteroides vulgatus* numbers), leading to a reduction in weight and adiposity, as well as improving systemic inflammation and lipid metabolism⁵⁴ (Table 1).

Gut microbiome transfer is an effective treatment for *Clostridium difficile* infection^{55,56}. However, Alang et al. reports an interesting case where a female patient with recurrent *C. difficile* infection developed obesity following gut microbiome transfer⁵⁷ (Table 1). Of note, it was reported that she had not lost any weight as a result of the infection, but developed new-onset obesity after receiving gut microbiome from a healthy but similarly overweight donor. This weight gain was likely due to both resolution of her infection and alteration of the gut microbiome with transfer of obesogenic bacteria. Further, Van Nood et al. showed an increase in bacterial diversity in patients with *C. difficile* infection treated with gut microbiome transfer that was similar to that of healthy donors after donor-faeces infusion⁵⁸. The restoration of normal gut microbiome with transfer from a healthy donor may be further developed into targeted therapy for treating other conditions including obesity.

Do antibiotics lead to long-term alterations in the gut microbiome?

Recent studies in adults (using 16S rRNA gene sequencing) have shown that antibiotics are able to alter the intestinal microbiome in the short term by reducing the diversity of bacterial taxa⁵⁹⁻⁶⁴. The long-term effects of antibiotics on gut microbial composition have been more variable, with studies reporting differences in both inter- and intra-individual responses to the same antibiotic^{62,65}.

Although there is a high prevalence of antibiotic use in children, there are very few studies looking into their effect on the developing gut microbiome. A recent longitudinal study of the gut microbiome in 39 children showed fairly similar findings as in adults⁶⁶. This study analysed stool samples of children that had been exposed or unexposed to multiple courses of antibiotics within the first 3 years of life. Using both 16S rRNA gene and metagenomics sequencing, it was reported that there was less microbial diversity in terms of bacterial species and strains at 3 years of age in the children exposed to antibiotics⁶⁶.

However, there were apparent flaws in the studies published so far, as the number of subjects were small and had significant demographic heterogeneity^{63,67}. The type of antibiotic used, its route of administration, and the subject's age at the time of administration seem to influence the effects on the gut microbiome^{64,68,69}. In addition, diet, lifestyle, other medications, and products such as probiotics further complicate this conundrum^{53,70,71}.

Do antibiotics cause obesity?

Antibiotics have been linked to a dysbiotic gut microbiome, which in turn have been proposed to lead to obesity. The mechanisms by which antibiotics modulate weight gain are unclear, but several hypotheses have been proposed^{4,72}, which include: (i) increased ability from gut bacteria to extract energy from indigestible polysaccharides; (ii) reduction in the number of bacteria that are metabolically protective against obesity; (iii) altered hepatic lipogenesis; (iv) altered metabolic signalling; and (v) reduction in intestinal defence and immunity.

Since the 1950s, antibiotics have been added to food and water in pigs, cows, and chickens as an effective method to enhance growth, especially weight gain⁷³. This practice has been widely adopted by farmers, and a number of different antibiotics have been used for this purpose⁷⁴⁻⁷⁶. Notably, early life exposure to antibiotics in these animals has far greater effects on weight gain and feed efficiency than if exposure occurred in later life⁷⁴.

Not surprisingly, evidence from animal models have shown a direct link between treatment with low doses of antibiotics and changes in body composition^{15,77}. In 2012, Cho et al. demonstrated that administration of sub-therapeutic doses of antibiotics increased adiposity in young mice and also altered the intestinal microbiome⁷⁷ (Table 2). Two years later, Cox et al. observed that there was a critical period around the time of birth when mice were particularly vulnerable to low-dose antibiotic exposure¹⁵ (Table 2). Male mice whose mother were treated with penicillin before birth and throughout weaning exhibited markedly increased total mass and fat mass¹⁵. In contrast, both male mice receiving antibiotics after weaning and female mice receiving antibiotics before birth and after weaning had similar body composition when compared with controls¹⁵ (Table 2). The authors also reported that low doses of antibiotics enhanced the effects of a high-fat diet on the development of obesity in these mice¹⁵. Additionally, the study examined possible obesogenic effects of antibiotic-induced alterations in the gut microbiome. Caecal microbiota was transferred from 18-week-old control and penicillin-treated mice to 3-week-old germ-free mice, and those that received the penicillin-altered microbiota were heavier and had greater fat mass compared with controls¹⁵ (Table 2).

There have been a number of studies that aimed to assess whether the use of antibiotics may lead to obesity in humans, which are summarized in Table 2. In adults, small observational studies reported that subjects treated with either broad- or narrow-spectrum antibiotics were more likely to gain weight compared with those who were not treated⁷⁸⁻⁸⁰. However, among the limitations of these studies were heterogeneous populations (e.g. different baseline body mass index [BMI]) and small number of subjects. Nonetheless, an interventional study by Mikkelsen et al. demonstrated that treating 12 lean and healthy male subjects for 4 days with a combination of vancomycin, gentamicin, and meropenem reduced the gut bacterial population but was not associated with changes in postprandial glucose tolerance, insulin secretion, or plasma lipid concentrations⁸¹ (Table 2). There was also a slight increase in body weight of 1.3 kg and an acute but reversible increase in peptide YY secretion⁸¹ (Table 2). These effects might not be as pronounced compared with those of a longer antibiotic therapy, but a limitation of this study was the lack of a control (placebo) group.

In children, the evidence linking antibiotics usage to development of obesity has been inconsistent (Table 2). Prophylactic antibiotic treatment in children with cystic fibrosis was shown to result in weight gain⁸² (Table 2). Data from 10 randomised controlled trials indicated that antibiotic use in prepubertal children from low and middle-income countries leads to increased growth, particularly weight gain⁸³ (Table 2). However, these studies have only examined children with ongoing infections, whose resolution with antibiotic therapy might have been the underlying cause of weight gain, rather than the possible alterations in the gut microbiota.

Seven recent epidemiological studies in healthy children have provided evidence that the use of antibiotics is associated with obesity⁸⁴⁻⁹⁰ (Table 2). These studies reported that antibiotic exposure in early life was linked to increased BMI and a greater prevalence of obesity among healthy children⁸⁴⁻⁹⁰. This effect was more marked in children who were: i) treated within the first 6 months of life; ii) exposed to three or more courses of antibiotics; iii) treated with broad-spectrum antibiotics; and iv) males^{84-88,90} (Table 2). Notably, the available evidence indicates that the associations of both gut microbiome and antibiotic usage with childhood obesity are often sexually dimorphic. These studies have shown that the adverse effects seen in males are not often mirrored in females. This is not particularly surprising as sexual dimorphism in association with environmental stressors early in life have been well described in the literature⁹¹, even though the underlying mechanisms remain poorly defined. It is possible for example, that such dimorphism may result from differential adaptive responses to diet, physical activity, and physiological stress^{91,92}. Clearly, the evidence shows that observations made on one sex cannot be readily extrapolated to the other.

A very recent meta-analysis of 15 studies by Shao et al. did find that early life antibiotics exposure significantly increases risk of childhood obesity⁹³ (Table 2). Unfortunately, as all these studies were observational in design, it is difficult to pinpoint antibiotic usage as the primary cause of obesity without considering other confounding factors, such as host genetics, dietary changes, maternal BMI, breastfeeding, maternal smoking, and the infection itself^{84,85,88,89,94}. Some of the data in these studies were also potentially unreliable as they were based on recall of antibiotics usage, and compliance with the prescribed antibiotic therapy was never reported^{84,87,89,95}.

In contrast, two studies in 2016 suggest that there was no evidence that antibiotics had an effect on weight gain or on the development of obesity in children^{95,96}. The first study showed no effects of prolonged antibiotic prophylaxis (trimethoprim-sulfamethoxazole) for 2 years on weight gain in children with recurrent urinary tract infection⁹⁶ (Table 2). However, this study used a single class of antibiotics as prophylaxis, so that the results might have been affected by the type and dose of antibiotics used. Gerber et al. reported on a large retrospective longitudinal study of nearly 39,000 children, finding that antibiotic exposure within the first six months of life did not affect weight gain between 2 and 5 years of age⁹⁵ (Table 2). Nonetheless, as with other previous studies, there was inadequate information regarding antibiotic exposure and compliance with the prescribed therapy.

Does infection cause obesity?

Alternatively, infection rather than antibiotics could be the contributing factor to the development of obesity. A large study on over 260,000 individuals reported an association between infection and increased odds of obesity in childhood and adolescence, which was independent of antibiotic treatment⁹⁴ (Table 2). Further, the odds of obesity were also higher in the group with untreated infections than among those who were both uninfected and untreated (Table 2). A limitation in that study was that the assessment of obesity was made by comparing BMI among children across a wide age range (from 2 to 18 years), and comparisons between children of similar age might have yielded different results.

If infection is said to cause obesity, should we also consider viruses and other pathogenic organisms apart from bacteria as causative agents for obesity? This concept 'infectobesity' is not new and has been studied in animal models for the past two decades⁹⁷. At least ten different viruses have been reported to cause obesity in animals, including canine distemper virus, SMAM-1 avian adenovirus, and human adenoviruses Ad36 and Ad37⁹⁸. Further, SMAM-1 and Ad36 in particular, have been found to be associated with obesity in humans^{97,98}. However, beyond associations, the role of these viruses in the development of obesity in humans remains unclear.

Conclusion

There are strong data supporting the role of antibiotics in the development of obesity in well-controlled animal models, but evidence in humans remains inconclusive. Association studies suggest young children may be particularly vulnerable to the weight-promoting effects of recurrent courses of antibiotics through alterations in the gut microbiome. Randomised controlled trials could clarify the effects of long-term antibiotic treatment on weight gain. However, it would be unethical to conduct such studies, and we will need to rely on trials of antibiotic exposure in children where weight gain is a secondary outcome. These studies would help us better understand the role of antibiotics in the ongoing obesity epidemic.

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Table 1. Published evidence on a link between gut microbiome and obesity.

AAA, aromatic amino acid; AMPK, phosphorylated AMP-activated protein kinase; ANGPTL4, angiopoietin-like protein 4; BCAA branched chain amino acid; BMI, body mass index; CI, confidence interval; hsCRP, highly-sensitive C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; RYGB, Roux-en-Y surgery; SG, sleeve gastrectomy; WHR, waist to hip ratio.

STUDY	POPULATION	EXPOSURE AND DURATION	FINDINGS	COMMENTS
Backhed et al. 2004 ²⁸	C57BL/6J mice Donors: conventionally-raised mice Recipients: germ-free mice	Transplantation of germ-free mice with microbiota from caecum of conventionally-raised mice	↑ 60% body fat ↑ leptin, glucose, insulin ↑ insulin resistance ↓ ANGPTL4 in gut epithelium	There was increased body fat and insulin resistance within 14 days, despite reduction in food intake.
Backhed et al. 2007 ²⁹	C57BL/6J germ-free mice	Exposure to a Western-style (high-fat and high-sugar diet)	Maintains lean body phenotype due to: (i) ↑ levels of phosphorylated AMP-activated protein kinase (AMPK) and its downstream targets in skeletal muscle and liver (ii) ↑ ANGPTL4	This was due to increased fatty acid metabolism.
Osto et al., 2013 ³⁰	Wistar rats	Roux-en-Y surgery (RYGB) or sham surgery	Compared to sham rats, RYGB rats had: ↓ weight ↑ total bacterial content in alimentary limb and common channel ↑ <i>Bifidobacterium</i> spp., <i>Bacteroides</i> spp., and <i>Prevotella</i> spp. in common channel, alimentary limb, and colon	Bariatric surgery induced changes in the gut microbiome of rats to resemble those seen after prebiotics treatment or by dieting.
Tumbaugh et al. 2006 ³¹	C57BL/6J mice Donors: obese (ob/ob) or lean (+/+) mice Recipients: germ-free mice	Transplantation of caecal microbiota from obese or lean mice to germ-free mice	↑ percentage body fat in mice colonised with obese microbiota compared with lean microbiota	The gut microbiome of obese mice has an increased capacity for energy harvest and is transmissible.
Tumbaugh et al. 2008 ³²	C57BL/6J mice	Transplanting caecal microbiota from obese or lean mice to germ-free mice fed a CHO diet.	↑ % body fat in mice colonised with microbiota from obese (43% vs 25%), despite no difference in initial weight or food consumption of recipients.	Gut microbiome transfer from obese mice to germ-free mice resulted in a significant increase in adiposity compared with transfer from lean mice. Other experiments in the study showed that dietary modifications may also have an effect on the gut microbiome, and in turn on weight gain and adiposity.
Alang & Kelly 2015 ⁵⁷	Donor: one overweight healthy woman (daughter) Recipient: one overweight woman with <i>Clostridium difficile</i> infection (mother) (USA)	Gut microbiome transfer from daughter to mother to treat <i>C. difficile</i> infection	After 16 months: ↑ BMI +7.0 kg/m ² ↑ weight +34 pounds (15.4 kg)	Treated recipient's BMI increased from 26 to 33 kg/m ² after 16 months, despite attempts to halt weight gain including dieting and engagement in physical activity. Gut microbiome transplant may result in the development of obesity.
Liu et al. 2017 ³⁵	Exp 1: Adults (China) n=151	Exp 1: Metagenomic sequencing and metabolomics profiling on obese and lean (controls)	Exp 1: In obese individuals: ↓ gene counts and ↓ bacterial diversity	Alterations in obesity-associated gut microbiome were linked to metabolic changes which are associated with weight loss, reduction in adiposity and possibly improvement in obesity-related metabolic parameters.

STUDY	POPULATION	EXPOSURE AND DURATION	FINDINGS	COMMENTS
	<p>Mean age of 23 years</p> <p>Exp 2: C57BL/6 male mice</p> <p>Exp 3: Adults (China) who had sleeve gastrectomy (SG)</p>	<p>individuals</p> <p>Exp 2: Male mice fed a standard diet or a high-fat diet (HFD), then divided into 3 groups gavaged with live <i>Bacteroides thetaiotaomicron</i> (<i>Bthet</i>), sterile saline, or heat-killed <i>B. thetaiotaomicron</i> for 7 weeks.</p> <p>Exp 3: Metagenomic sequencing and metabolomics profiling on obese individuals post sleeve gastrectomy (SG). Controls: lean individuals (from Exp.1)</p>	<p>↑ <i>Ruminococcus torques</i>, <i>R. gnavus</i>, <i>Dorea longicatena</i>, <i>D. formicigenerans</i>, <i>Coprococcus comes</i>, <i>Lachnospiraceae</i> bacterium, <i>Fusobacterium ulcerans</i>, and <i>F. varium</i></p> <p>↑ Firmicutes</p> <p>↓ Bacteroidetes/Firmicutes ratio</p> <p>↓ <i>Bacteroides thetaiotaomicron</i>, <i>B. uniformis</i>, <i>B. xylanisolvens</i>, <i>B. ovatus</i> and <i>Bacteroides</i> spp.</p> <ul style="list-style-type: none"> pronounced differences in 148 metabolites, including 20 of 34 amino acids (e.g. glutamate, phenylalanine, tyrosine, and aromatic and branched-chain amino acids) glutamate had highest increase in obese individuals, and it was inversely correlated with <i>B. thetaiotaomicron</i> numbers. higher glutamate levels associated with obesity traits (e.g. ↑ BMI, ↑ waist circumference, ↑ HOMA-IR, ↑ hsCRP and, ↑ triacylglycerol). <p>Exp 2: Mice on standard diet + live <i>Bthet</i>: ↓ total and ↓ inguinal fat mass</p> <p>Mice on HFD + live <i>Bthet</i>: prevention of further weight gain and adiposity ↓ expression of genes encoding proteins involved in lipogenesis. ↑ expression of genes involved in lipolysis and fatty acid oxidation ↓ levels of inflammatory markers</p> <p>Exp 3: Gut microbiome of obese individuals after SG changed to resemble that of lean individuals, particularly with: ↑ <i>B. thetaiotaomicron</i> numbers ↓ serum glutamate ↓ hyperglycemia, ↓ insulin resistance, ↓ leptin, and ↓ inflammatory markers</p>	
Nicolucci et al. 2017 ⁵⁴	<p>Children who were overweight or obese (Canada)</p> <p>n=42</p> <p>Ages 7 to 12 years</p>	<p>Treatment with oligofructose-enriched inulin (OI) for 16 weeks</p> <p>Control: maltodextrin</p>	<p>OI-treated group had:</p> <ul style="list-style-type: none"> ↓ body weight z-score (-3.1%) ↓ percentage body fat (-2.4%) ↓ percentage trunk fat (-3.8%) ↓ interleukin-6 (-15%) ↓ serum triglycerides (-19%) 	<p>Randomized controlled, double-blind, placebo-controlled trial</p> <p>Authors showed that the positive effects of prebiotic supplementation were associated with alteration in the gut microbiome (mainly ↓ <i>Bacteroides vulgatus</i> and ↑ <i>Bifodabacterium</i> spp.), which in turn resulted in reductions in weight, adiposity, systemic inflammation, and an improvement in lipid profile.</p>
Ridaura et al. 2013 ³³	<p>Donors: human twin pairs discordant for weight (USA)</p> <p>Recipients: C57BL/6J germ-free mice</p>	<p>Experiment 1: Oral transplantation of gut microbiota from obese or lean human twins to germ-free mice</p> <p>Experiment 2: Co-housing the above mice 5 days after transplantation</p>	<p>1: ↑ weight gain in mice transplanted with gut microbiota from obese twin</p> <p>2: Co-housing mice harbouring the obese twin's microbiota with mice containing the lean twin's microbiota soon after gavage prevented the development of obesity</p>	<p>Mice are coprophagous, allowing for cross-transfer of microbiome between co-housed individuals. Gut microbiome transfer might have an effect on body composition and obesity.</p>

STUDY	POPULATION	EXPOSURE AND DURATION	FINDINGS	COMMENTS
Sanchez et al. 2014 ⁴⁴	Adults who were overweight or obese (Canada) n=125 Ages 18 to 55 years	Supplementation with <i>Lactobacillus rhamnosus</i> CGMCC1.3724 (LPR) for 24 weeks Controls: placebo Each group was submitted to moderate energy restriction for first 12 weeks (phase 1) followed by weight maintenance for subsequent 12 weeks (phase 2).	Phase 1: ↓ weight (-1.8 kg) and ↓ fat mass (-1.2 kg) in LPR-treated women no differences among men Phase 2: ↓ weight (-2.6 kg) and ↓ fat mass (-2.5 kg) in the LPR-treated women no differences among men	Randomised double-blind, placebo-controlled trial. Probiotic supplementation led to a greater reduction in weight and fat mass in women, but not in men.
Sharafedinov et al. 2013 ⁴⁵	Adults with metabolic syndrome (Russia) n=40 Ages 30 to 69 years	Normal diet supplemented with probiotic cheese or standard cheese (controls) for 3 weeks	↓ body mass index (BMI) in the probiotic cheese group	Randomized, double-blind, placebo-controlled, parallel pilot study. Probiotics may influence weight reduction.
Takahashi et al. 2016 ⁴⁶	Adults who were overweight or obese (Japan) n= 160 Ages 20 to 65 years	Milk supplemented with <i>Bifidobacterium animalis</i> ssp. <i>lactis</i> GCL2505 for 12 weeks Control: unsupplemented milk	↓ visceral fat area (VFA) in <i>B. lactis</i> group at 8 weeks (-6.8 cm ² ; 95% CI -10.6,-2.9) and 12 weeks (-5.1 cm ² ; 95% CI -8.6,-1.5).	Randomised double-blind, placebo-controlled trial. <i>B. lactis</i> led to a reduction in visceral fat, which is associated with metabolic disorders.
Tremaroli et al. 2015 ³⁴	Donors: obese women who had RYGB, vertical banded gastroplasty, or no surgery (Sweden) Recipients: germ-free Swiss Webster female mice	Transplantation of gut microbiota from women 9.4 years post-bariatric surgery or women who did not have any surgery to germ-free mice	↓ fat deposition by 43% in mice transplanted with microbiota post-RYGB and by 26% post-VBG compared with mice colonized with microbiota from women with no surgery	Bariatric surgery seems to alter gut microbiome that are involved in metabolism and regulation of adiposity.
Zhang et al. 2016 ⁴⁷	Adults n=1934 Ages ≥ 18 years	Probiotics consumption	↓ weight by 0.59 kg (95% CI 0.30,0.87) ↓ BMI by 0.49 kg/m ² (95% CI 0.24,0.74) Greater BMI reduction: • with multiple species of probiotics • with ≥8 weeks treatment • in overweight/obese individuals	Meta-analysis of 20 studies. Probiotics consumption may have an effect on weight reduction and adiposity.

Table 2. Existing evidence for a link between antibiotic use and obesity.

aOR, adjusted odds ratio; BMI, body mass index; BMI SDS, body mass index standard deviation score; CI, confidence interval; Exp, experiment; LDP, low-dose penicillin, OR, odds ratio; RR, relative risk.

STUDY	POPULATION	EXPOSURE	FOLLOW-UP	FINDINGS	OBESITY ASSOCIATION?	COMMENTS
Cho et al. 2012 ⁷⁷	C57BL/6J mice	Sub-therapeutic antibiotic treatment at weaning (age 3 weeks) through life Controls: no antibiotics	Life-long	Compared to controls, the antibiotic group had: ↑ total fat mass ↑ 3% body fat ↑ glucose-dependent insulintropic polypeptide (GIP) ↑ relative concentrations of Firmicutes bacteria compared to Bacteroidetes ↑ short-chain fatty acids ↑ lipogenesis and triglyceride synthesis	Yes	Administration of sub-therapeutic antibiotic therapy altered the gut microbiome and increased adiposity in mice.
Cox et al. 2014 ¹⁵	Exp.1,2,3: C57BL/6J mice Exp.4: germ-free Swiss Webster mice	Exp.1: Antibiotic treatment (low-dose penicillin [LDP]) at birth or at age 4 weeks and lasting throughout life [controls had no antibiotics] Exp.2: LDP lifelong with a high-fat diet at 17 weeks [4 groups – all combinations with and without LDP and/or high-fat diet] Exp.3: LDP during first 4 weeks, first 8 weeks, or lifelong with a high-fat diet at 6 weeks [controls had no antibiotics] Exp.4: Transferring antibiotic-treated gut microbiota to germ-free mice	Life-long	Exp.1: ↑ weight if LDP administered at birth rather than at age 4 weeks, with greater effect in male mice Exp.2: ↑ fat mass in both male and female mice ↑ fasting insulin levels in male mice Exp.3: ↑ total, lean and fat mass in all groups, with greater effect in female mice Exp.4: ↑ total and fat mass in recipients of gut microbiome from penicillin-treated mice	Yes	LDP exposure from birth and in early life can alter metabolism in mice and lead to increased adiposity. LDP enhances the effect of high-fat diet on the development of obesity. The obese phenotype due to LDP-induced microbiome alterations is transferrable.
Ajslev et al. 2011 ⁸⁴	Children from the Danish National Birth Cohort n=28,354 Age 7 years	Antibiotic treatment up to 6 months of age Controls: no antibiotics	7 years	↑ risk of overweight among children of normal weight mothers (OR 1.54; 95% CI 1.09, 2.17) ↓ risk of overweight among children of overweight mothers (OR 0.54; 95% CI 0.30, 0.98) ↓ risk of overweight among children of obese mothers (OR 0.85; 95% CI 0.41, 1.76)	Yes / No	Prospective longitudinal study. Antibiotics introduced early in life may have a differential effect on childhood obesity risk, which may vary according to maternal BMI.

STUDY	POPULATION	EXPOSURE	FOLLOW-UP	FINDINGS	OBESITY ASSOCIATION?	COMMENTS
Angelakis et al. 2014 ⁷⁹	Adults with Q fever endocarditis (France) n=82 Ages 40 to 70 years	Long-term treatment (at least 18 months) with doxycycline and hydroxychloroquine Controls: no antibiotics	1 year after completion of treatment	↑ weight in 23% of antibiotics-treated patients (+2 to +13 kg) ↓ weight in 6% of antibiotics-treated patients	Yes / No	Case control study. Treatment with doxycycline and hydroxychloroquine combined was associated with both weight gain and weight loss.
Azad et al. 2014 ⁸⁵	Children from the Canadian Birth Cohort n=723 Assessments at 9 and 12 years of age	Antibiotic treatment during the first year of life Controls: no antibiotics	9 and 12 years	↑ risk of overweight if they received antibiotics in the first year of life (32.4% versus 18.2% at age 12) ↑ risk of overweight more marked in boys (aOR 5.35; 95% CI 1.94, 14.72).	Yes	Case control study. At age 12 years, children (but particularly boys) who received antibiotics in the first year of life were more likely to be overweight compared with those who were unexposed ⁸⁶ .
Bailey et al. 2014 ⁸⁶	Children (USA) n= 65,480 Age 24-59 months	Antibiotics prescribed in infancy (ages 0-23 months) Controls: no antibiotics	Up to 5 years	↑ risk of obesity with cumulative exposure to antibiotics (≥4 courses) (RR 1.11; 95% CI 1.02, 1.21) ↑ risk of obesity with exposure to broad-spectrum antibiotics (RR 1.16; 95% CI 1.06, 1.29) ↑ risk of obesity with early exposure (0–5 months of age) to broad-spectrum antibiotics (RR 1.11; 95% CI 1.03, 1.19), but lesser with exposure at 6–11 months of age (RR 1.09; 95% CI 1.04, 1.14)	Yes	Retrospective cohort study. This large study has provided evidence that antibiotic treatment in the first 2 years of life was associated with obesity in early childhood.
Edmonson & Eickhoff 2016 ⁹⁶	Children enrolled in the Randomized Intervention for Children With Vesicoureteral Reflux Study (USA) n=607 Median age of 12 months (range from 2 months to 5.9 years of age)	Treatment with trimethoprim-sulfamethoxazole for 2 years Controls: placebo	2 years	No difference in weight gain (weight-for-age Z-scores) between groups at 6, 12, 18, or 24 months after treatment initiation No effect on the prevalence of overweight/obesity at 24 months (Antibiotic 24.8% vs Placebo 25.7%)	No	Secondary analysis of data from a randomized clinical trial. Prolonged (2-year) prophylactic treatment with trimethoprim-sulfamethoxazole had no effect on weight gain or overweight/obesity risk in young children with recurrent urinary tract infection.
Francois et al. 2011 ⁸⁰	Adults referred for routine upper endoscopy (USA) n=69 Ages 50 to 78 years	Patients positive for <i>Helicobacter pylori</i> infection were treated with a 14-day regimen of amoxicillin, clarithromycin, and a proton pump inhibitor Controls: no antibiotics	18 months after completion of treatment	↑ BMI (+5%) in patients treated for <i>H. pylori</i> Compared to pre-treatment levels: ↑ post-prandial ghrelin levels (near 6-fold increase) ↑ leptin levels (+20%)	Yes	Prospective cohort study. <i>H. pylori</i> eradication was associated with increased ghrelin and leptin levels (both are involved in appetite and energy regulation) as well as an increase in BMI.

STUDY	POPULATION	EXPOSURE	FOLLOW-UP	FINDINGS	OBESITY ASSOCIATION?	COMMENTS
Gerber et al. 2016 ⁹⁵	Children from 30 paediatric primary care practices (USA) n=38,522 singleton and 92 twins (46 matched pairs) Age 7 years	Antibiotic use in the first 6 months of life Controls: no antibiotics	7 years	<ul style="list-style-type: none"> No significant difference in weight gain between 2 and 5 years of age (0.7%; 95% CI -0.1, 1.5%) No significant weight difference in twin pairs discordant in antibiotics exposure (-0.09 kg; 95% CI -0.26, 0.08 kg) 	No	<p>Retrospective, longitudinal study of singleton births and matched longitudinal study of twin pairs.</p> <p>There was no association between antibiotics use in early life and changes in weight in early childhood.</p> <p>The analysis on twin pairs reduces the potential environmental and genetic factors that may modify weight gain, but statistical power was reduced due to a small sample size.</p>
Gough et al. 2014 ⁹⁴	Children from 7 low or middle-income countries n=4,316 children Ages 1 month to 12 years	Oral antibiotic treatment Controls: no antibiotics or placebo	Mean of 268 days	<ul style="list-style-type: none"> ↑ weight by 23.8 g/month (95% CI 4.3, 43.3) ↑ height by 0.04 cm/month (95% CI 0.00, 0.07) 	Yes	<p>Meta-analysis of 10 randomised controlled trials.</p> <p>Antibiotic treatment of prepubertal children from low and middle-income countries leads to increased growth, particularly weight gain.</p>
Li et al. 2016 ⁹⁴	Children from the Kaiser Permanente Northern California population (USA) n= 260,556 Age 2 to 18 years	Antibiotic treatment or infection within the first year of life 3 groups: <ul style="list-style-type: none"> Children with infection treated with antibiotics Children with infection but not treated Controls (no infection and no antibiotics) 	Up to 18 years	<ul style="list-style-type: none"> No association between antibiotic use during infancy with risk of childhood obesity (OR 1.01; 95% CI 0.98, 1.04) ↑ odds of childhood obesity in children with infection compared with controls (OR 1.25; 95% CI 1.20, 1.29) 	No	<p>Longitudinal birth cohort study.</p> <p>Infection rather than treatment with antibiotics during infancy was associated with increased odds of childhood obesity.</p>
Mikkelsen et al. 2015 ⁸¹	Healthy adult men (Denmark) n=12 Ages 18 to 40 years	4-day treatment with combination of oral vancomycin, gentamicin, and meropenem	6 months	<ul style="list-style-type: none"> ↑ body weight (+1.3 kg) ↑ peptide YY secretion ↓ gut bacterial population No changes in postprandial glucose tolerance, insulin secretion, or plasma lipid concentrations 	Yes	<p>Interventional study.</p> <p>A short course of antibiotics had an effect on gut microbiome population and was associated with some weight reduction, but had no effect on glucose or lipid metabolism.</p>
Murphy et al. 2014 ⁸⁷	Children from 18 different countries involved in the International Study of Asthma and Allergies in Childhood Phase Three n=74,946 Age 5 to 8 years	Antibiotic treatment in the first 12 months of life Controls: no antibiotics	Not mentioned	↑ BMI in boys with antibiotics treatment (+0.11 kg/m ²)	Yes	<p>Population-based cross-sectional study (secondary analysis from a multi-centre, multi-country, cross-sectional study).</p> <p>Treatment with antibiotics during the first year of life is associated with a small increase in BMI in boys but not in girls in mid-childhood.</p>

STUDY	POPULATION	EXPOSURE	FOLLOW-UP	FINDINGS	OBESITY ASSOCIATION?	COMMENTS
Saari et al. 2015 ⁸⁸	Children from a Finnish birth cohort n=12,062 Age ≥24 months	Antibiotic treatment before the age of 24 months Controls: no antibiotics	Not mentioned	↑ BMI SDS with antibiotic treatment (boys +0.13 SDS; girls +0.07 SDS) compared to controls More pronounced effect with: • exposure to macrolides before 6 months of age (boys +0.28 SDS; girls +0.23 SDS) • >1 exposure (boys +0.20 SDS; girls +0.13 SDS).	Yes	Population-based study. Antibiotic treatment in the first 2 years of life was associated with increased BMI SDS in healthy children. However, the apparent effect was exacerbated according to type, timing, and frequency of treatment.
Saiman et al. 2010 ⁸²	Children with cystic fibrosis (USA and Canada) n=260 Ages 6 to 18 years	Treatment with azithromycin for 3 days per week over 24 weeks Controls: placebo	24 weeks	↑ weight (+0.58 kg; 95% CI 0.14, 1.02) in antibiotic-treated group	Yes	Multi-centre, randomized, double-blind placebo-controlled trial. Antibiotic treatment was associated with a slight weight gain in children with cystic fibrosis.
Scott et al. 2016 ⁹⁰	Children in The Health Improvement Network (UK) n=21,714 Age 4 years	Antibiotic treatment before 2 years of age Controls: no antibiotics	4 years	↑ odds of obesity at 4 years (OR 1.21; 95% CI 1.07, 1.38) ↑ odds of obesity with repeated exposures: • 3–5 prescriptions (OR 1.41; 95% CI 1.20, 1.65) • ≥6 prescriptions (OR 1.47; 95% CI 1.19, 1.82)	Yes	Retrospective cohort study. Antibiotic treatment in the first 2 years of life was associated with increased odds of obesity at age 4 years, which were higher with increased exposure.
Shao et al. 2017 ⁹³	Children from developed countries n=445,880	Early life antibiotics exposure (prenatal to 23 months) Controls: no antibiotics	2 to 20 years	↑ risk of childhood overweight (RR 1.23; 95% CI 1.13, 1.35) ↑ risk of childhood obesity (RR 1.21; 95% CI 1.13, 1.30) ↑ BMI z-score (mean difference 0.07 95% CI 0.05, 0.09) For every additional course of antibiotic exposure: ↑ 7% risk of overweight (RR 1.07; 95% CI 1.01, 1.15) ↑ 6% risk of obesity (RR 1.06; 95% CI 1.02, 1.09)	Yes	Systematic review and meta-analysis of 15 studies Early life antibiotics exposure significantly increases risk of childhood obesity.
Thuny et al. 2010 ⁷⁸	Adults with suspicion of infective endocarditis (France) n=96 Ages 45 to 77 years	4 treatment groups: 1) gentamicin + vancomycin, 2) gentamicin + amoxicillin, 3) other antibiotics, and 4) controls [no antibiotics]	6 weeks	↑ BMI in patients treated with any antibiotics (+1.1 kg/m ²) compared to controls ↑ BMI in patients treated with gentamicin + vancomycin (+2.3 kg/m ²)	Yes	Case-control study. Antibiotic treatment may have an effect on weight gain.
Trasande et al. 2013 ⁸⁹	Children from the Avon Longitudinal Study of Parents and Children Cohort (ALSPAC; UK) n=11,532 Assessments at 6 weeks, 10 months, 20 months, 38 months, and 7 years of age	Antibiotic treatment in the first 2 years of life Controls: no antibiotics	Up to 7 years	Antibiotic treatment before 6 months of age: ↑ weight-for-length Z-scores at 10 and 20 months (+0.11 and +0.08, respectively) ↑ BMI Z-score at 38 months (+0.07) ↑ odds of overweight at 38 months (OR 1.22)	Yes	Prospective longitudinal study. Antibiotic treatment in the first 6 months of life was associated with increased odds of overweight throughout early childhood.

Figure 1: Proposed roles of the gut microbiome on obesity.

ANGPTL4, angiopoietin-like protein 4; FXR, nuclear farnesoid receptor X receptor; GLP-1, glucagon-like peptide; LPS, lipopolysaccharide; SCFA, short-chain fatty acids; and TGR5, G-protein coupled bile acid receptor 1.

