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Abstract

In recent years, science has made great strides in its understanding of the microbiome, discovering that it plays a role in regulating many body processes. One area of study is the microbiomes interaction and influence on host metabolic processes. Studies using both mice and humans have established a clear correlation between obesity and the composition of the microbiome, identifying a microbiome unique to obese individuals. Furthermore, experiments with germ-free mice have shown that the microbiome effects host metabolism, causing germ free mice to increase in mass when inoculated with normal microbiota. Inoculation with microbiota from obese mice yielded greater increases in mass, showing the obesogenic effect of the microbiota. The mechanisms through which the microbiome can contribute to obesity are enhanced extraction of energy from food, and increased capacity for nutrient uptake in the gut, and alteration of metabolic pathways by suppressing fasting induced adipose factor and decreasing AMPK activity. Many of these pathways show increased activity in obese mice. The enhanced energy extraction coupled with greater deposition of fat mediated by altered metabolic pathways can contribute to obesity.

The role of the microbiota in obesity, combined with decades-old observations that antibiotics, particularly early in life, increased the weight of livestock, led to a hypothesis that antibiotics can disrupt the development of the microbiome, causing metabolic changes, leading to obesity. Recently, this hypothesis has been tested, both in studies utilizing mice, and in many epidemiological studies. This paper will evaluate the available evidence to determine if exposure to antibiotics early in life can lead to increased incidence of obesity later.

Introduction

In 1671 when Antony van Leeuwenhoek first spied "animalcules," now known to us as single cell organisms, through his homemade microscope, he set into motion a centuries long scientific endeavor to discover, describe, catalogue and gain a deeper understanding of the vast microbial world that surrounds us. This laid the groundwork for the development of the germ theory by Louis Pasteur and Robert Koch over the course of the 1860's and 1870's. Germ Theory, the theory that many diseases are caused by microbial agents, revolutionized medicine leading to many advances such as the disinfecting of wounds, the appreciation of the need for a sterile environment during surgery, and in 1921 the fortuitous discovery of penicillin by Alexander Fleming. Germ theory also had a profound influence on the attitudes of medicine and society at large towards "germs." Although only a tiny fraction of microbes are pathogenic, they became indelibly associated, both in the popular imagination and in medical practice, with sickness and disease. This focus on microbe pathogenicity has yielded tremendous benefits to public health, in fact antibiotics were among the primary drivers of rising life expectancy in developed countries in the mid twentieth century (Armstrong, 1999), but antibiotics, for all their benefits, came with costs as well, namely their effect on the native flora.

Discovery of the body's native flora began in the mid 1880's when an Austrian pediatrician, Theodore Escherich, observed the eponymously named Escherichia coli in the stool of healthy children. Discovery continued apace and the realization set in that there was a large, diverse, community of microbes that colonized the skin, nasal and oral cavities, and the urogenital and gastrointestinal tracts of healthy people, making up their native flora. As early as the 1970's there was already an idea of the number of microbes, then estimated to number roughly 10^{14}, living primarily in the gastrointestinal tract, and some idea of ecology of this diverse community (Savage, 1977). Until relatively recently, it was assumed that the microbes living in the colon lived a largely commensal existence, dining on food indigestible to the human host but not interacting with the host in any meaningful way. With this understanding collateral damage to the gut bacteria because of antibiotics was no great concern, with the worst-case scenario being an unwanted bloom of C. Difficile. However, in recent years and particularly since the launch of the Human Microbiome Project, an entirely different picture has emerged, one that includes many symbiotic relationships between the native flora and the host, in fact so enmeshed is the host-symbiont relationship, that they have been described as one "supraorganism (Turnbaugh, et al., 2007). This new understanding demands a closer look at the possible effects of antibiotics on our microbiome.

This paper will explore the possible link between antibiotics and obesity. First it will lay the groundwork with a thorough exploration of the literature linking the state of the microbiome to obesity as well as an understanding of the underlying mechanisms. Then it will consider the evidence that a disturbance of the microbiota through antibiotic treatment can cause obesity along with proposed causative mechanism. Finally, it will propose ways to mitigate the effects of the antibiotic treatment.

Methods

Information for this paper was obtained primarily through online searches utilizing google scholar as well as well as numerous databases accessed through Touro college's library system.

Definition of "Core Microbiome"

The first step to determining if obesity is associated with an altered microbiome is establishing the baseline values defining what a "normal" gut microbiome looks like. This task is complicated by the wide diversity of gut microbial populations found across different geographical areas and cultures, and even within communities and cultures. In fact, in one study "there was not a single abundant (defined as >0.5% of the community) bacterial species shared by all 154 individuals" involved in the study (Turnbaugh P. J., 2009) . However, if one looks at the microbiome on the level of phyla, a strong pattern begins to emerge, with bacteria from the phyla Firmicutes and Bacteriodetes representing, in one study, 92.6% of the microbiota (Ley, et al., 2006). These 2 phyla and their respective ratios can serve as one definition of a core microbiota.

Another way of defining the core microbiome is, rather than focusing on the species or phyla present, focusing on the various genes present. Various studies utilizing this methodology have found that regardless of the vast diversity of the microbial makeup of the gut flora, there exists a "wide array of shared microbial genes, comprising an extensive, identifiable 'core microbiome' at the gene, rather than at the organismal lineage, level" (Turnbough, et al., 2009). While both definitions are useful, this paper will primarily utilize the second definition of the microbiome as a set of genes rather than ratio of different bacterial phyla. The reason for this is that a focus on genes can better illuminate any products of the microbiome that may affect host metabolic pathways, possibly contributing to obesity.

Association of Obesity with Altered Microbiome

Having established a baseline microbiome, we can now explore any obesity associated changes that may occur. First, we will explore the changes in the ratio of Firmicutes and Bacteroidetes associated with obesity.

In one experiment, mice heterozygous for obesity ob/+ (due to a defective leptin gene) were mated producing litters consisting of a mix of obese (ob/ob) and lean (ob/+ and +/+) phenotypes. Microbial ecology in the gut, specifically the ratio of Firmicutes to Bacteroidetes, which is typically similar among members of a family living together, was found to be consistent in the heterozygous mothers as well as the ob/+ and +/+ children. In the homozygous ob/ob mice of the same litter however, a sharp increase in abundance of Firmicutes relative to Bacteroidetes was observed (Ley, et al., 2005). This shows a clear correlation between obesity and the composition of the gut microbiome.

In another experiment, 12 obese individuals were randomly placed on either a fat or carbohydrate restricted diet and their gut microbiota was monitored over the course of a year for any discernible shift in the microbiota as they lost weight. Initially, obese people had more firmicutes and fewer Bateroidetes than lean controls. However, over the course of the year as their weight dropped, the ratio of Firmicutes to Bacetoidetes began to more closely resemble a typical lean profile (Ley, et al., 2006).

Together, these two studies, encompassing both mice and men and showing both an increase in abundance of Firmicutes as an obese phenotype was acquired in the mouse experiment and a decrease in its abundance as weight was lost in the human experiment, firmly establish that obesity is associated with an altered microbiome.

Can the Gut Microbiome Cause Obesity?

Having established a strong correlation between obesity and altered gut microbial ecology, we can now explore the possibility that the microbiome can be a causative agent in obesity.

There seems to be good experimental evidence, at least with mice, that this is the case.

In one experiment, mice were divided into three groups. One group was raised "Germ Free" meaning that their gut was sterile. Another group consisted of regular, conventionally raised mice, and acted as a control. A third group was initially raised germ, free but subsequently inoculated with gut bacteria at 7-10 weeks by spreading a suspension of cecal content from the control group mice on their fur. Comparison of germ free and regular mice at 8 to 10 weeks found that regular mice had 42% more body fat than their germ-free companions and had epidydimal fat pads weighing 47% more, all while eating 29% less food. After a 14-day colonization, a process known as conventionalization, the third group of mice experienced a dramatic 57% increase in total body fat and a 61% increase in epididymal fat pad weight all while their chow consumption decreased to that of the normal mice (Backed, et al., 2004). This experiment shows that gut microbiota has a powerful effect on metabolism and fat storage. The initial low-fat state of the germ-free mice even with their above average food intake and their dramatic increase in fat, even in the face of decreased chow consumption, as they were conventionalized, indicates that a normal microbiome plays a key role in regulating fat in mice.

Having established the effects of a normal microbiome, let us examine the effects of an obese one. Toward this end, an experiment was constructed in which germ-free mice were colonized by gavage (meaning they were fed by tube) with the cecal contents of either wild type +/+ or genetically obese ob/ob (leptin deficient) mice. In the 14-day period following the colonization food consumption in the (ob/ob) and (+/+) transplanted groups was not statistically different (55.4±2.5g for ob/ob against 54.0±1.2g for

+/+) and they both ate the same type of chow (no difference in caloric density). Despite this, the mice colonized with ob/ob microbiota exhibited a significantly greater increase in body fat than those colonized with +/+ microbiota with the ob/ob colonized mice increasing body fat by 47±8.3% and the +/+ colonized mice increasing by just 27±3.6% (Turnbaugh,et al., 2006).

The dramatic difference in body fat between the two groups strongly indicates that the obese microbiome causes greater adiposity, and gives rise to the possibility that the microbiome can play a role in its development.

Mechanisms of Microbiome Influence on Adiposity

The classic, somewhat simplistic understanding of the development of obesity is to take the calories of the food eaten, subtract calories burned by both the basal metabolic rate and any additional energy expenditures for various activities, and assume that the remainder is stored as fat in adipose tissue throughout the body. Our exploration of the mechanisms through which the gut microbiota increase adiposity will illuminate several ways that this seemingly straightforward and commonsense equation can be altered.

One mechanism proposed is that all microbiomes increase the bodies capacity for energy harvest from food eaten by excreting exoenzymes that break down polysaccharides that the host is unable to metabolize. Once degraded into monosaccharides and short chain fatty acids, both the bacteria and the host readily take up the product, accruing extra calories to the host from the same food. It is further hypothesized that the changed obese microbiome performs these tasks more efficiently extracting even more calories from the same unit of food with the host reaping some of the benefits. This hypothesis is buttressed by numerous lines of evidence.

The first of these is a simple comparison of the energy remaining in the feces of regular mice as opposed to genetically obese mice. Bomb calorimetry showed that ob/ob mice have significantly less energy remaining than regular mice, yielding 3.2 kcal/g compared to 3.4 in regular mice (Turnbaugh, et al., 2006). This is simple, clear, empirical evidence that an obese microbiome harvests more energy than a standard, lean microbiome.

Another line of evidence involves a genetic analysis of the microbiome, specifically of genes encoding enzymes that catalyze the breakdown of polysaccharides indigestible to their hosts. In one study, a sequencing of 18 Human microbiomes identified genes for 156 carbohydrate- active enzymes, which are enzyme families that break down carbohydrates, including 77 glycoside hydrolase, 21 carbohydrate-binding module, 35 glycosyltransferase, 12 polysaccharide lyase and 11 carbohydrate-esterase families. These genes consisted of fully 2.62±.013% of all the microbial genes sequenced, a higher percentage than any other identified group of genes. Furthermore, an analysis of lean and obese twins found that the obese twins had a microbiome that was significantly enriched in genes coding for carbohydrate, lipid, and amino acid metabolism as compared to that of their lean twins (Turnbough, et al., 2009).

Mice studies have yielded similar results, with ob/ob mice having microbiomes containing more genes coding for various carbohydrate-active enzymes as compared with their lean littermates. A predicted result of this would be an increased concentration of the products of bacterial fermentation of these polysaccharides, such as butyrate and acetate, in the cecum of the ob/ob mice. This prediction was borne out, with cecal butyrate concentration of obese mice double those of lean mice and acetate levels 20% higher (Turnbaugh, et al., 2006).

Interestingly this same study also found a greater abundance of archaea in the obese mice than their lean counterparts. Archaea oxidize the hydrogen produced as a by-product of fermentation by gut bacteria, turning it into methane. By removing a product of the fermentation reaction, they increase its efficiency, serving to further enhance energy extraction by the obese microbiome. Indeed, in a study of mice colonized with archaea commonly found in the human gut, Methanobrevibactor smithii and B. thetaiotamicron, a significant increase in the efficiency of bacterial polysaccharide fermentation leading to an increase in adiposity in the mice was observed (Samuel & Gordon, 2006)

In addition to increasing energy extraction from food, there is also evidence that the microbiome increases the hosts capacity for uptake of nutrients in the gut. In one experiment, germ-free and conventionalized mice were fed a glucose solution. After fifteen minutes, the level of glucose uptake was found to be twice as high in the conventionalized mice as in the germ-free ones (Backed, et al., 2004). Additionally, the microbiome is essential to the development of the capillary network to transfer these nutrients from the intestines to the hepatic portal vein. Germ-free mice were found to have arrested development of this capillary network, and upon conventionalization, developed it to normal levels within ten days (Stappenbeck & Hooper, 2002).

These lines of evidence collectively paint a picture of a microbiome that extracts more energy from food by breaking down complex polysaccharides that the host is unable to metabolize on his own and amplifying the hosts ability to absorb the resultant monosaccharides, providing one possible mechanism for the microbiome to contribute to obesity.

Another mechanism proposed is that the microbiome modifies cell signaling pathways to increase fat storage, that is, to direct more of the energy harvested toward adipocyte storage rather than other metabolic functions. Two metabolic pathways are involved, one of which involves fasting-induced adipose factor (Fiaf) which is a lipoprotein lipase inhibitor (Backed, et al., 2004). Lipoprotein lipase facilitates deposition of fat in adipocytes. Fiaf, which inhibits it, is a crucial regulator of this process. Fiaf is produced by brown and white fat, the liver, and the intestine. The microbiome suppresses the production of Fiaf in the intestinal epithelium, thereby increasing the activity of lipoprotein lipases, resulting in more triglycerides being incorporated into adipocytes.

Experimental evidence for this machanism comes from a study that compared regular germ-free mice, germ-free mice incapable of producing Fiaf (Fiaf-/-), and conventionalized mice both with and without the Fiaf gene. The regular germ free mice, as expected were the leanest. Germ free Fiaf-/- mice however, were found to have nearly the same amount of fat as their conventionalised wild type peers. Furthermore, a conventionalization of the germ free Fiaf knockout mice yielded a minimal increase in body fat of 10±8% versus 55±16% for the wild type germ free mice. Conventionalization of heterozygotes (Fiaf +/-) yielded an intermediate result, consistant with the hypothesis. Additionally comparison of mRNA of conventionalised and germ free wild type mice revealed camparitively less Fiaf expression in the small intestines of the the former, expression elswhere though was unaffected (Backed, et al., 2004). Other studies have had similar findings, including one that found that while regular germ free mice were resistant to obesity induced by consuming an "american diet" in their case chow with higher fat content and more easily digested sugars, Fiaf -/- mice had lost this resistance (Backhed, et al., 2007). These findings point to fasting induced adipose factor as a major component of the microbiomes contribution to adiposity.

Another pathway involves levels of AMP-activated protein kinase, or AMPK (Backhed, et al,. 2007). AMPK is a key enzyme regulating metabolism, serving as the lynchpin of a complex web of metabolic pathways maintaining proper ATP levels. AMPK ramps up energy production in response to metabolic stresses. It is triggered primarily, as its name indicates, by an increased ratio of AMP to ATP, but also by numerous other factors such as an elevated ratio of NAD to NADH, and the hormones leptin and adiponectin (Kahn, et al,. 2005) . The microbiome is thought to decrease AMPK activity, leading to lower energy expenditure, with more calories remaining to be deposited as fat.

Evidence for this mechanism is based on a number of observations. The first is that germ free mice were found, using an immunoblot assay, to have phospho-AMPK, which is the active form, at concentrations 40% percent greater than their regular peers in their gastrocnemeus muscles. Consistant with these findings AMP levels in the germ-free mice were foud to be 50% higher. Additionally, many other enzymes involved in the fatty acid oxidation pathway triggered by AMPK showed fluctuations consistant with increased fatty acid oxidation. In this pathway, AcetylCoA carboxylase converts Acetyl CoA to Malonyl CoA, Malonyl CoA inhibits carnitine-palmitoyl transferase-1 (Cpt1), which catalyzes the rate-limiting step for uptake of long chain fatty acids by mitochondria, AMPK phosphorylates AcetylCoA carboxylase, inhibiting it and thereby increasing fatty acid oxidation (Kahn, et al, 2005). A 43% increase in the levels of phosphorylated AcetylCoA carboxylase was found using an immunoblot assay and a 17% increase in the level of Cpt1 was found with a biochemichal assay, in germ free over that of regular mice,both consistant with increased fatty acid oxidation (Backhed, et al,. 2007).

Collectively, these lines of evidence paint a picture of a microbiome that acts on both sides of the energy equation, harvesting more energy from food through greater polysaccharidase activity, conserving more of that energy through, and depositing a greater portion of it as fat.

Can Antibiotics Contribute to Obesity?

Having gained some appreciation of the influence of our microbiota on our metabolism and its role in promoting adiposity, we can now explore the role of antibiotics on this complex system. Specifically, we will explore whether the disruption to the microbiota caused by antibiotics, particularly early in life, can affect the body mass of the host later in life by either promoting or inhibiting weight gain.

There is extensive evidence that antibiotics promote weight gain, from veterinary medicine, animal models, and epidemiological studies.

In the 1950's Veterinary scientists showed that giving pigs (Taylor & Gordon, 1955) and other livestock (Jukes & Williams, 1953) sub-therapeutic doses of antibiotics increased their growth causing them to gain more weight without increasing feed consumption. It subsequently became common practice among farmers to mix low doses of antibiotics into the feed of pigs, cows, sheep, and poultry, increasing their weight, a practice that continues to this day. The effect on weight gain is significant, a meta-analysis of numerous studies gauging the weight boosting effects of adding antibiotics to feed in pigs found an increase in weight gain of up to 15% and an increase in feed efficiency (an industry term for amount of meat produced per unit of feed) of up to 6%. The strongest effects were found when the antibiotics were given from birth with lesser, though still significant effects

found if they were given at later dates (Hays, 1969). Evidence that the weight gain is connected to disruption of the microbiota comes from experiments with germ free chickens. Germ free chickens given feed containing antibiotics that had a growth promoting effect on regular chickens showed no similar increase in growth from the treatment (Coates, 1963), indicating that the weight gain is a result of modulation of the microbiome. These veterinary studies on regular and germ free animals, combined with the everyday experience of farmers for the last 70 years provide one line of evidence that antibiotic use has a role in weight gain. Importantly, as the data showed, the effect is greatly magnified in early life, suggesting that the early microbiome may be particularly vulnerable to whatever disruption causes the weight gain, a theme that will be expanded on shortly.

More evidence comes from experiments with model organisms, namely Mus Musculus, the mouse. Additionally, these experiments shed light on the magnified effects of antibiotics found in early life.

In one experiment the effects of sub-therapeutic antibiotic treatment (STAT) was tested on mice to attempt to replicate its observed effect on farm animals and gain some insight into its mechanisms. In the experiment mice were exposed, starting at weaning, to various common antibiotics by putting them in their drinking water at sub-therapeutic levels, and were compared to a control group using various metrics. After a seven-week exposure, the STAT mice were found to have greater fat mass than the control group as well as a significantly higher percent body fat. Curiously although fat mass was greater in the STAT mice, total mass was not significantly greater at seven weeks, though later measurements taken from 8 to 26 weeks did show increased mass in the STAT mice (Cho, et al., 2012).

Utilization of other metrics allows for a deeper understanding of the processes driving the adiposity of the STAT mice. One important measure taken was the level of Gastric Inhibitory Polypeptide (GIP). GIP, a hormone secreted by K cells in the small intestine, stimulates lipoprotein lipase activity, increasing fat storage and contributing to adiposity (Miyawaki, et al., 2002). GIP levels were found to be substantially elevated in STAT mice (39.1±2.5 pg/ml) compared to the controls (24.4±4.2 pg/ml). This provides a possible mechanism for the observed increase in adiposity.

Microarray analysis of differential gene expression in hepatic tissue yielded deeper insights into the metabolic changes wrought by STAT. Comparison of STAT and control mice found upregulation of pathways for lipogenesis and triglyceride synthesis in the STAT mice, further contributing to adiposity.

Examination of the gut bacteria in the STAT mice yielded further insight. Although the overall number of bacteria did not change significantly, the composition of the microbiome did change, with the abundance of Firmicutes increasing relative to that of Bacteroidetes, which, as discussed earlier, is typical of obese microbiomes. Additionally, examination of the cecal contents of the STAT mice found higher levels of butyrate and acetate, suggesting increased energy capture through fermentation of complex carbohydrates indigestible to the mice, as discussed earlier. Supporting evidence came from metabolic cage experiments showing no difference in caloric intake but a lower caloric output in fecal pellets in STAT mice compared to controls (Cho, et al., 2012).

Taken together, these measurements paint a picture of antibiotics changing the composition of the microbiome, leading to metabolic changes causing adiposity and weight gain, and suggest that perhaps antibiotics can contribute to obesity in humans as well.

Increased Effect in Early Life

Greater weight gain was observed in farm animals when STAT was started earlier in life. Mouse studies have explored the importance of the timing of antibiotic exposure further, experimentally confirming these observations and expanding upon them. They found that early life is a critical time in metabolic development and exposure to antibiotics at this sensitive stage can permanently alter host metabolism.

Evidence for these claims comes from an experiment comparing mice started on low dose penicillin (LDP) at weaning (LDP-w) to mice where LPD was started shortly before birth(LDP-b) so that the initial colonization with maternal microbiota would be altered. A control group was maintained that was not exposed at all.

The experiment found that earlier administration of antibiotics did have amplified effects. The growth rate for LDP-b was greater than the control, the fat mass as well as the total mass of adult LDP-b male mice was greater than that of LDP-w mice and the control (Cox, et al., 2014), demonstrating enhanced effects of earlier antibiotic administration. Sexual dimorphism was apparent in the results with the females experiencing lesser if any effects, a finding that remains unexplained. Metabolic differences between the LDP-b and LDP-w mice were found as well with the LDP-b mice having greater expression of genes involved in adipogenesis than LDP-w mice.

The mammalian early microbiome is a dynamic, changing environment typically showing a pattern of succession as different taxa first dominate then diminish (Pantoja-Feliciano, et al., 2013). Altered representation of some of these taxa has been

associated with obesity (Kalliomaki, et al,. 2008). Typically, Lactobacillus is prominent in nursing animals, as was indeed the case with the controls. The LDP-b mice however, showed much lower levels of Lactobacillus, as well as other groups whose population typically peak in early life such as Candidatus Arthomitus and Allobaculum (Cox, et al., 2014). Although the precise roles of these microbes are not known, their suppression by LDP and the dramatic phenotypic effects that follow suggest some role in metabolic development.

In an experiment with worrying implications for human obesity, some LDP mice were switched to high fat diet at 17 weeks, and were compared to control groups with just a high fat diet or just LDP. The growth promoting effects of LDP were accentuated by the high fat diet producing fat and weight gain surpassing that produced by the high fat diet or LDP alone.

More worrying still, the metabolic effects of LDP lasted into adulthood even after treatment finished. Mice that received LDP for only four weeks after birth still experienced greater fat and total mass accumulation from 6-20 weeks. This weight gain persisted even though the microbiota had appeared to normalize.

Finally, to demonstrate that the metabolic and phenotypic changes observed were a result of an altered microbiota and not some direct effect of the penicillin, cecal microbiota were transferred from 18-week-old control and LDP mice to 3 week germ-free mice. The mice inoculated with the LDP microbiota increased total mass and fat mass at a faster rate (.078 g/day total mass and .058 g/day fat mass faster) than those inoculated with the normal microbiota (Cox, et al., 2014).

These studies provide convincing evidence, as much as can be inferred from model organisms, that antibiotics contribute to obesity through disruption of the microbiota, and that early life is a particularly sensitive time when disruption of the developing microbiome can have long lasting metabolic effects.

Epidemiological Studies

The findings in model organisms that antibiotic exposure, particularly in early life, can lead to obesity, have important implications for human health. However, results in model organisms do not always translate into results in humans. Since ethical concerns preclude the types of randomized, controlled studies routinely performed with model organisms from being done on humans, we must rely on epidemiological evidence. Fortunately, there are a wealth of well-constructed epidemiological studies demonstrating that antibiotic exposure in infancy is correlated with obesity later in life.

In a study involving 28354 mother baby pairs from the Danish National Birth Cohort, antibiotic exposure in the first six months of life was correlated (with an odds ratio of 1.54, well above the threshold for showing correlation) with an increased risk of being overweight at 7 years (Ajslev, et al., 2011). Supporting these findings are results from a study utilizing 11532 children from the Avon Longitudinal Study of Parent and Children. This study examined antibiotic exposure during three early-life time windows, 6< months, 6-14 months, and 15-23 months. Exposure under six months was, once again, strongly correlated ($p<.001$) with increased body mass at 10, 20, and 38 months. However, exposure from 6-14 months showed no effect and exposure at 15-24 showed a modest weight gain at 7 years (Trasande, et al., 2013). A Canadian study combining data from health records and a Canadian longitudinal birth cohort study further bolstered these findings. The study found that children who received antibiotic treatment in the first year of life were more likely to be overweight at ages 9 and 12 than their untreated peers (32.4% overweight if exposed vs. 18.2% if not). Additionally, researchers noted a greater prevalence of elevated central adiposity, a precursor of metabolic syndrome, among the treated children (Azad, et al, 2014). A longitudinal study in the USA (Bailey, et al., 2014)and a global cross sectional study (Murphy, et al., 2013) found similar results as well. Several the studies (Trasande, et al., 2013) (Murphy, et al., 2013) found a strong sexual dimorphism, with the effect much greater in boys, and nearly all the studies showed some difference between girls and boys, with the boys seeing greater weight gain than the girls, a finding that while replicated many times in both model organisms and in humans, has not been satisfactorily explained.

The collective weight of these epidemiological studies gives great credence to claims that antibiotics contribute to obesity.

Mitigating the Effects of Antibiotics

Even with all the evidence of detrimental side effect of antibiotics, stopping their use is obviously not an option. Antibiotics are a cornerstone of modern medicine, without which life expectancy would surely drop precipitously. However perhaps a bit of restraint in prescribing antibiotics to children is in order. Rates of antibiotic prescriptions in the USA are unnecessarily high, with some analyses finding that fully half of all antibiotic prescriptions written are unnecessary (Nyquist, et al., 1998). Although antibiotic prescription rates among children and adolescents have dropped since that finding (Lee, et al., 2014), prescription rates in the USA are still high compared to some other countries. In Sweden, for example, antibiotic use is 53% lower than in the USA (Ternhag & Hellman, 2010). This indicates that prescription levels can still be lowered significantly without adversely effecting public health. While some antibiotic exposure may be unavoidable for many children, even merely reducing the

number of rounds of antibiotics they take reduces their chance of developing obesity later in life (Bailey, et al., 2014).

One final action possible to mitigate the obesogenic effects of antibiotics on children is to prescribe narrow spectrum antibiotics when possible. One study, despite finding significant correlation between broad-spectrum antibiotics in the first two years of life, found no such correlation for narrow-spectrum antibiotics (Bailey, et al., 2014).

Conclusions

In conclusion, the evidence for an obesogenic effect of early-life exposure to antibiotics is substantial and convincing. The many mouse studies demonstrating the influence of the microbiome on metabolism and its role in obesity give ample reason to suspect that perturbations of the microbiome with antibiotics may have some effect on obesity. The evidence from farm animals, mice, and epidemiological studies serve to confirm that suspicion, showing that antibiotic exposure in infancy contributes to one's chances of developing obesity later in life.

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