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Original Research

Effect of Antibiotic Administration during Infancy on Growth Curves through Young Adulthood in Rhesus Macaques (*Macaca mulatta*)

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Recent human studies indicate a possible correlation between the administration of antibiotics during early life and the risk of later obesity, potentially due to antibiotic-induced alteration of the gastrointestinal microbiome. In humans, the risk of obesity increases with multiple courses of antibiotics and when fetuses or infants are treated with broad-spectrum and macrolide antibiotics. In addition, the obesity risk in humans seems higher for males than females. We used a retrospective, case-control, matched-pair study design to evaluate health records for 99 control-matched pairs of rhesus macaques (*Macaca mulatta*) from an outdoor breeding colony. We hypothesized that NHP treated with antibiotics prior to 6 mo of age would have steeper growth curves than those who were not. However, in contrast to prior research with humans and mice, growth curves did not differ between antibiotic-treated and control animals. Differences between humans and NHP may have influenced this outcome, including the relative standardization of NHP environmental factors and diet compared with those of human populations, types of infections encountered in infancy and choice of antibiotic treatment, and the different relative maturity at 6 mo of age in the 2 species. The results provide support for current standard medical practice in NHP and highlight a difference between macaques and humans that may influence future obesity research using macaques. Determining the basis for this difference might improve our understanding of the risks of early-life antibiotic treatment and suggest mitigation strategies for treating infant illnesses without risking obesity.

Research since the 1940s has shown that administering substances with antibiotic properties during the first few months of life to pigs, chickens, and cattle can affect the growth pattern in these food animals.[11,20,22] This association was noted even before it was clearly understood that the compounds being administered had antibiotic properties (many were given to increase access to vitamins in diets).[12] In addition, early-life antibiotic exposure has been shown to significantly influence body composition and growth rate of mice.[10] Recent clinical research has supported the hypothesis that exposure to particularly broad-spectrum antibiotics[1,37,45] or macrolide antibiotics[37] in humans younger than 12 mo of age increases the likelihood of obesity,[30,39] particularly with repeat exposures.[5] This effect may begin prior to birth in humans; one study found an 84% higher risk of obesity at age 7 when mothers had taken antibiotics in the second or third trimesters of pregnancy.[41]

In the early 20th century, evidence of potential harm from overuse and misuse of antibiotics began to accumulate.[9,19] At the same time, evidence for unexpected positive effects emerged, such as the prevention of the onset of diabetes in nonobese diabetic mice given vancomycin[29] and the use of specific types of antibiotics as effective treatments for noninfectious disorders such as hepatic encephalopathy and irritable bowel syndrome.[13,43] Since the initiation of the current study in late 2014, the volume of available human literature regarding the effect of early antibiotic administration on obesity and overweight status has increased markedly, thus helping to highlight outstanding questions and possible refinements for future research. The 'obesity epidemic' is a key focus of current research, and the effects of obesity on economics[18] and quality of life are wide-reaching. Although obesity has a genetic component, the explosion of overweight status and obesity within the current world population over the past 70 y has been multifactorial. Evaluation of concurrent changes in living standards for possible obesity triggers previously focused on changes in agricultural and eating practices, but another important change occurring within the relevant time frame was the development and subsequent widespread use of antibiotics. The exposure of late-term fetuses and very young children to antibiotics is high, in no small part due to increases in C-section rates over the past 30 y, from approximately 21% of all United States births in the mid1990s to more than 32% in 2014, the last year for which full data were available.[27] Antibiotics are standardly administered to mothers undergoing C-sections prior to infant delivery, thus exposing the fetus,[44] with exposure in the perinatal period reported to be as high as 45% of all deliveries.[34]

Human infants exposed to antibiotics develop different gastrointestinal microbiota than infants not exposed to antibiotics.[1,5,45] Documented differences exist between the gut flora in obese compared with lean human populations[24,41,42] as well as in obese and lean mice.[24] In mice, the microbiome content has been shown to have important contributions to energy balance: for example,

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germ-free mice consume more calories but have a lower body fat percentage than conventionally reared cohorts. However, when germ-free animals are inoculated with microorganisms from the distal bowel of their conventionally reared cohorts, there is a rapid increase (57%) in total body fat content without concurrent increase in caloric consumption.[4] The composition of the human microbiome flora is established during the early developmental period and can be affected by many factors, including gestational age at birth, type of delivery (vaginal delivery compared with Cesarean section), feeding method (nursed compared with bottle-fed infants), and antibiotic administration during the early stages of life.[17] The processes and mechanisms by which altering the microbiome affects growth are not completely understood, but antibiotics have conclusively been shown to dramatically alter the gastrointestinal microbiome of both humans and mice.[10,17,21,31,45] The composition of the intestinal flora directly affects energy balance in mice; energy harvest from dietary sources, storage of energy in the form of triglycerides, and fatty acid oxidation leading to energy expenditure have all been demonstrated experimentally to modify energy balance in mice.[4,31,42] Alterations of this equation can mediate downstream metabolic effects, including diet-induced obesity, insulin resistance, and diabetes.[18,21,23,31] Prior research regarding the microbiome of NHP has focused primarily on its characterization, its social and immunologic factors, and the influence of neonatal dietary choices,[2,6,32,40,46] but the current study is the first to study the effect of antibiotic use on the growth of NHP.

The aim of our study was to determine the effect of antibiotic administration on the growth curves of rhesus macaques (*Macaca mulatta*) through early adulthood. Understanding this effect will help to evaluate the potential use of macaques as models for the effects of antibiotics on growth and potentially will aid understanding of the mechanisms involved in the increased risk of obesity associated with antibiotic administration in humans. In addition, the current findings will help to clarify the potential research implications of standard antibiotic use in infant NHP with enteric bacterial disease or trauma, because macaques are a common model in obesity research. The hypothesis was that early antibiotic administration (that is, before 6 mo of age) would cause increased growth in NHP by age 5 y, similar to the increased growth in humans and mice that results in obesity.

Materials and Methods

Animal selection and study design. All animals were born and housed at the Oregon National Primate Research Center (Beaverton, OR), an AAALAC-accredited facility. All animal procedures were IACUC-approved and were conducted at this facility in accordance with Public Health Service Policy on the Humane Care and Use of Laboratory Animals and with the Animal Welfare Act.

We used a retrospective case-control matched-cohort study design to compare the growth rates of rhesus macaques from birth through age 5 y for animals that received antibiotics prior to 6 mo of age with those who did not. Study animals were matched according to sex and birthdate—each macaque that received antibiotics was matched with another of the same sex that had a similar birthdate but that had not received antibiotics. One year was chosen as the outside limit to restrict the potential for marked husbandry and diet alterations yet to not exclude animals unnecessarily. Birthdates were matched as closely as possible, within 20 ± 74 d for males and 49 ± 247 d for females. Our study compared each macaque with its pair animal only, and both animals in each pair received near-identical housing, diets, and medical care; this design made the study a more variable-controlled evaluation of the true effect of antibiotics than is possible with human studies. Human studies must control for factors including socioeconomic factors, maternal smoking, breastfeeding compared with formula diets, and race or ethnicity, giving our study an advantage regarding the sample size necessary to control for variables. Records from 1984 to 2014 were reviewed. Similar to some human studies in the early stages of this type of research,[3,5,30,39] the type of antibiotic and duration of administration were not limited: any animal that received any antibiotic prior to the 6-mo time point was included in the antibiotic group.

Inclusion criteria for the study were: 1) survival to at least 5 y of age; 2) housed in an outdoor breeding group for no less than 4.5 y of the first 5 y of their life; and 3) no fewer than 5 weights documented between birth and 5 y of age. The animals included spanned a 30-y time frame: the earliest birth date included was 1984, and the most recent animal reached 5 y of age in 2014. Information evaluated included animal age, sex, weights between birth and age 5 y, and whether animals were treated with antibiotics prior to 6 mo of age.

The subjects of the study were 99 matched pairs (87 pairs of female macaques and 12 pairs of males) of Indian-origin rhesus macaques .This sex ratio is representative of the outdoor housed population, and all animals meeting the criteria for the study were included. For at least 4.5 of the first 5 y of life, all macaques were housed in social groups of 30 to 250 animals in open-topped, natural-flooring (dirt and grass), 1-acre enclosures or in indoor–outdoor shelter-style housing with concrete flooring. These enclosures contained multiple climbing and play structures. All animals experienced natural light and weather conditions and had the option to move freely to and from huts within corrals and accessible indoor areas (heated during cold ambient temperatures) for shelter. Animal care staff monitored all areas every morning and reported any apparently ill or injured animals for veterinary evaluation. Macaques requiring care were brought to the colony hospital and released back to the enclosure once treatment was complete. Hospital housing is in standard NHP cages on a 12:12-h light:dark cycle, with a temperature range of 64 to 84 °F (17.8 to 28.9 °C). A standard Old World primate laboratory diet (currently Fiber-Balanced Monkey Diet 5000, Purina, St Louis, MO) was provided twice daily to all animals, and they were supplemented with fresh fruits and vegetables and drinking water without restriction. Macaques were weighed at least once yearly; animals that were brought to the hospital for treatment were weighed as a part of their veterinary evaluation and thus may have had more weight assessments per year.

Time spent in indoor caging reflects approximate days of illness, because outdoor-housed animals are brought inside temporarily to resolve illness or injury. The mean time spent inside for all animals on study in the first 5 y of life (that is, during the study period) was 23 d; the range was 0 to 139 d for female macaques and 0 to 104 d for males. The time spent inside roughly doubled for animals treated with antibiotics compared with those untreated (males: treated, 30.6 d; untreated, 15.8 d; females: treated, 34.0 d; untreated, 12.6 d), but all animals still spent relatively few days indoors during their first 5 y.

No animals included in this study suffered from chronic diarrhea (a common ailment of rhesus macaques) during the study

period, because the definition used in our facility would automatically disqualify animals from this project due to time spent inside on treatment. At our facility, 'chronic diarrhea' is defined in a standard operating procedure to create consistency. Patients with this diagnosis meet at least one of the following criteria: multiple episodes of diarrhea which either do not respond to medication or respond but where the animal is unable to be tapered off the medication without relapse; diarrhea accompanied by weight loss of greater than 15% within a 90-d period that does not respond adequately to treatment; or animals needing to be removed from a social group to manage diarrhea recurrence (a maximum of 3 removals within a 2-y period). Macaques typically are unable to receive medications while in a group setting and are not left in a group while experiencing ongoing diarrhea and weight loss. Because inclusion in this study required that participants could not have spent significant time outside the group setting, no animals in this study met the facility criteria for chronic diarrhea.

As is common for outdoor group-housed macaques, the vast majority of ailments requiring treatment with antibiotics during the study period (the first 5 y of the animal's life) were trauma-related. Occasional infectious diseases resulted in antibiotic administration to some animals, and some animals not obviously ill were nonetheless treated prophylactically with antibiotics because of exposure to a confirmed infectious disease determined to be a threat to the health of the group. Antibiotics administered included enrofloxacin, with a total of 49 courses administered among 99 macaques treated with antibiotics. The next most commonly administered antibiotic was azithromycin, with 33 courses, followed by penicillin G, with 23 courses. A handful of other antibiotics were administered, all fewer than 6 times over all animals. The number of antibiotic events is greater than the number of macaques on study because some animals received multiple antibiotics at one time or had multiple courses during their first 6 mo (Table 1).

Animals included on our study had much greater variable control compared with human studies on this subject. Specifically, whereas similar human studies do not control for the effects of diet, housing, or environmental exposures during the study period, our study design specifically controlled for these variables, matching animals with others highly likely to have an extremely similar upbringing.

Statistics. Group differences in body weight were assessed by using a multiple regression model as a function of age and antibiotic use. For each matched pair, the interaction between age and antibiotic use was added as a covariate to compare the slope of the regression lines for the control and antibiotic groups. A paired *t* test was used to compare overall rate of body weight change for the 99 pairs of regression slopes and the 1- and 5-y body weight estimates obtained from multiple-regression models for each matched pair, as described earlier. A *P* value of less than 0.05 was considered to be statistically significant, and data were described by using descriptive statistics, including means and standard deviations. Statistics were evaluated and graphs created by using Statistica 12 (Dell Software, Round Rock, TX).

Results

Growth rate did not differ ($P = 0.45$) between rhesus macaques treated with antibiotics in infancy and untreated controls (Figures 1 and 2). The average growth rate (slope) for control female macaques was 1.25 ± 0.22 kg annually, compared with 1.21 ± 0.17 kg yearly for antibiotic-treated females. The average slope for control male macaques was 1.39 ± 0.16 kg annually, and that for antibiotic-treated males was 1.48 ± 0.30 kg yearly. There were no significant differences in 5-y weight estimates between the groups treated with antibiotics (mean ± 1 SD; females, 6.92 ± 0.92 kg; males, 7.86 kg ± 1.47 kg) and the control groups (females, 6.82 ± 0.80 kg; males, 7.53 ± 0.66 kg; Table 2). *P* values associated with comparisons of growth rate and weight differences at ages 1 and 5 y did not indicate significant differences between treated and untreated animals for either sex. In addition, we did not detect a significant linear trend in growth rate according to the number of days spent inside.

Table 1. Antibiotics administered

	Total female	Total male	Total both sexes
Azithromycin	26	7	33
Enrofloxacin	44	5	49
Penicillin G	24	2	26
Cefazolin	4	1	5
Chloramphenicol	1	0	1
Metronidazole	4	2	6
Tetracycline	5	0	5
Trimethoprim-sulfamethoxazole	4	0	4

The total number of antibiotic exposures is greater than 99 because some animals received multiple antibiotics or multiple courses.

Discussion

The aim of this study was to evaluate the effect of early antibiotic use in NHP on growth between 0 and 5 y of age. Our study did not find statistically significant differences between the treated and untreated groups in growth rate or weight averages at sexual maturity. The results of this study represent an important and interesting difference from prior research published in humans or using mouse models, in which antibiotic administration in infancy clearly led to increases in weight, height, or BMI later in life. Our study supports the conclusion that the overall influence of early-life antibiotic administration on weight gain in our general population of breeding NHP is likely to be small or nonexistent. In addition, scientists working with NHP on growth studies may not need to consider antibiotic exposure early in life a potential confounding variable, in contrast to those working with animal models where antibiotics are known to induce obesity and accelerated growth. Furthermore, the lack of effect in our study contradicts findings from similar human and mouse studies.

A retrospective approach using the electronic medical records database provided a large dataset for evaluation and models the large cohort studies in humans routinely used to investigate similar types of information. Human-subject studies require large numbers of participants to control for factors irrelevant to our study, including socioeconomic status, race or ethnicity, breast-feeding compared with formula feeding, and maternal smoking. The level of variable control that we achieved in the current study is not possible in a human population study and is part of the reason that we can comment on the significance of the effect of antibiotics despite relatively smaller sample sizes than typically needed for similar human studies. In addition, because of the

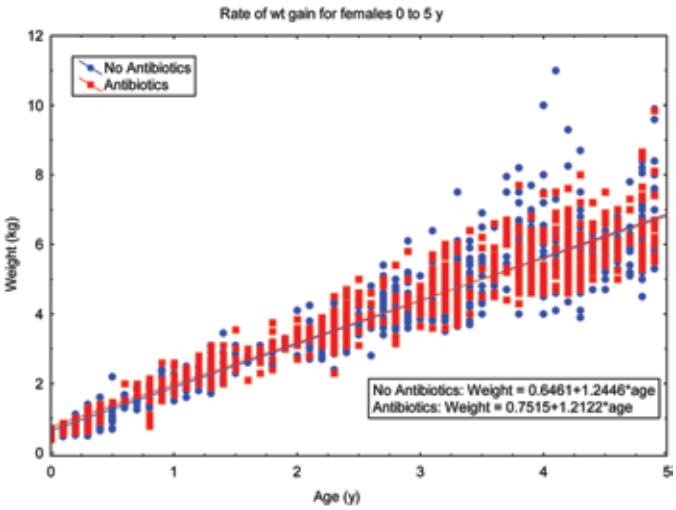

Figure 1. Rate of weight gain for female macaques between birth and 5 y of age. Weight gain in female macaques given antibiotics (red squares) prior to 6 mo of age did not significantly differ (P = 0.22) from that of untreated controls (blue circles); n = 88 per group.

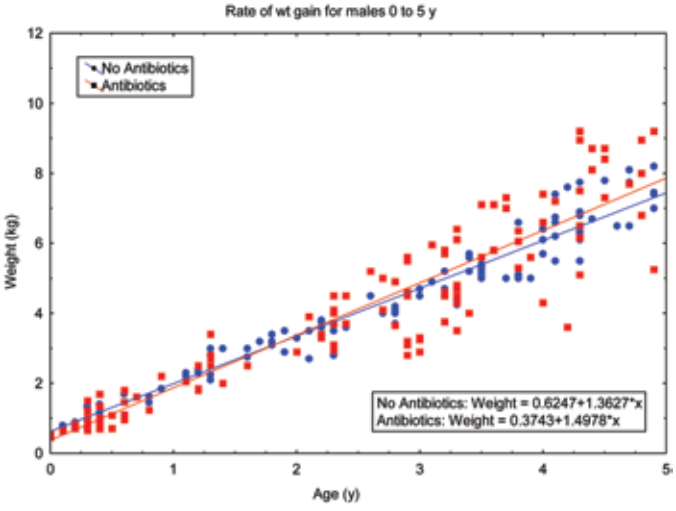

Figure 2. Rate of weight gain for male macaques between birth and 5 y of age. Weight gain in male macaques given antibiotics (red squares) prior to 6 mo of age did not differ significantly (P = 0.24) from that of untreated controls (blue circles); n = 13 per group.

breeding practices of rhesus macaques, where an alpha male is responsible for most or all of the pregnancies within his breeding group, the level of genetic relatedness within our study cannot be achieved in human studies. Although we do not have specific genetic information on animals within our study (the capability to monitor this parameter is too recent to have been possible for our project), this familial interrelatedness reduces variability due to factors other than the one being studied (early antibiotic administration).

By selecting macaques housed in an outdoor setting, we eliminated animals involved in invasive research projects that might alter growth rates during the timeframe of the study. In addition, outdoor housing ensured psychologic and nutritional consistency, because the animals were reared by their own dam. At our facility, environmental and husbandry practices are consistent among outdoor-housed animals, thus reducing variability in our study. The case-control design and selection of animals with near birthdates increased the likelihood that the 2 study groups experienced similar husbandry, veterinary care, and feeding practices. Statistically, providing each treated animal with a matched control reduces the effect of these potential variables and provides a significant advantage over human studies, where control of these types of variables is not reasonable.

We evaluated growth curves through age 5 y, when rhesus macaques typically have achieved sexual maturity.[14] Limiting inclusion to those animals that survived through age 5 y eliminates potential bias from infant mortality, because animals that die or are euthanized at a young age are more likely to have atypical growth rates prior to death. We used the number of days housed inside as a proxy for overall animal health. Restricting inclusion to only those animals housed outside for most (4.5 y) of their first 5 y reduces bias and weight growth skew due to significant illness, because young animals with significant illnesses typically remain indoors for care. In addition, both treated and untreated animals spent an average of fewer than 34 d inside, with the overall average for each sex being a total of 23 d spent inside. This result helps validate our limitation of days spent inside to reduce the confounding effect of significant illness on growth rate.

Several inherent differences between humans and NHP might have contributed to the rejection of our hypothesis: the relatively controlled environment and diets of NHP compared with humans, differences in standard antibiotic choices between NHP and humans, the types of infections requiring treatment at young ages, and the relative maturity of NHP when compared with human infants. In addition, the growth differences might occur early in life. Animals raised outside are weighed once or twice annually, and growth curves created from infrequent weights are necessarily more linear (especially during the rapid-growth phase, which typically lasts until approximately age 5 y) as a consequence. If temporary growth differences (that is, those lasting less than 1 y) in the control and antibiotic-treated populations are eroded by 'catch-up' growth, the infrequent weight measurements may not have captured those differences.

The nature of juvenile diseases experienced by the NHP population compared with the human population might have influenced the study outcome. Enteric disease and trauma are the most common diagnoses resulting in antibiotic treatment of infant macaques.[14] In contrast, most human infants who receive antibiotics at an early age do so as a consequence of otitis or upper respiratory infection.[1,4,45] Bacterial enteritis necessarily involves an alteration to the intestinal microbiome. Otitis media and upper respiratory disease seem less likely to alter the intestinal microflora (at least, until oral antibiotics are administered), given the anatomic separation.

Similar to many human studies, our evaluation did not consider either the length or number courses of antibiotics but rather used antibiotic exposure as a binary variable (exposed or not exposed). In human studies,[5] repeat exposures to antibiotics typically have been linked with increased BMI, although one study found no correlation.[15] Although the relative differences in frequency of use of fluroquinolones in juvenile NHP compared with humans might complicate comparison, some types of macrolides (specifically azithromycin, the second most-used antibiotic in this study's population) are commonly used in both species and could serve as the basis for additional research. In humans,

Table 2. Growth rates (mean ± 1 SD) in rhesus macaques

	Female (n = 88)			Male (n = 13)		
	Control	Antibiotic	P	Control	Antibiotic	P
Growth rate (kg annually)	1.25 ± 0.22	1.21 ± 0.17	0.22	1.40 ± 0.16	1.50 ± 0.29	0.34
Estimated weight at age 1 y (kg)	1.92 ± 0.22	1.98 ± 0.28	0.13	1.97 ± 0.25	1.94 ± 0.37	0.87
Estimated weight at age 5 y (kg)	6.92 ± 0.92	6.82 ± 0.80	0.50	7.53 ± 0.66	7.86 ± 1.47	0.48

Growth rates differed between sexes, as expected (rhesus are a sexually dimorphic species), but not between antibiotic-treated and nonantibiotic-treated groups. In addition, estimated weights at age 5 y differed between the sexes but not between treatment groups for either sex. Estimated weights at age 1 y did not differ between sexes, as expected, or between treatment groups for either sex.

broad-spectrum and macrolide antibiotics have been implicated as having the greatest influence on later BMI, growth rate, and gut microbiota,[19] whereas narrow-spectrum antibiotics, especially amoxicillin, did not have the same effect.[1,37,45] This difference may explain the lack of positive effects of antibiotics on growth rate in our current study. Still, recent research has shown that long-term low-dose penicillin administration results in a host of metabolic and fatty changes in mice,[25] and a longitudinal human study in the Netherlands found an association with both increased height and weight at age 10 to broad-spectrum β-lactam exposure (a class that in at least one study produced no appreciable change[15]), particularly multiple courses prior to age 2.[26]

The most commonly used antibiotic in our infant NHP, enrofloxacin (often administered for bacterial enteritis), is not used in human infants, and the analog, ciprofloxacin, is not a routine first-line therapeutic option.[44] This situation could be a possible factor in the differences between our study's findings and those of the human and mouse studies. Drugs metabolized by using the enterohepatic cycle (such as macrolides) allow the drug more direct contact with the colonic flora, compared with the renal metabolism of amoxicillin and cephalosporins, and may account for the increased effect of macrolides on BMI.[37] This hypothesis makes sense in light of recent evidence showing that, compared with IV administration, oral dosage of antibiotics stimulates increased antibiotic resistance, particularly for those drugs metabolized through the kidneys.[47] Fluoroquinolone antibiotics (including both enrofloxacin and ciprofloxacin) are dually metabolized in both the liver and kidneys,[35] and may therefore have less effect than macrolides but more than narrow-spectrum antibiotics such as the penicillins. Fluoroquinolones have been shown to create long-term changes and a reduction in bacterial diversity in the intestinal microbiome.[19]

In addition, several studies involving human subjects yield contradicting information regarding the timing of antibiotic exposure and its effect on later weight or BMI. Various timelines of exposure have been evaluated, but in general, antibiotic use within the first 6 mo of life is considered to have a correlation with obesity later in life.[1,5,37,39] One large cohort study found an effect on BMI when antibiotics administered between 15 and 23 mo of age.[39] In multiple studies, a persistent effect in boys was documented beginning at age 7 y until as old as 12 y.[1,3,5,30] Another study found a similar result of early antibiotic use on central adiposity, known to be an important risk factor for future health concerns, including metabolic and cardiovascular disease.[3] This association has been documented in a mouse model as well.[7] Furthermore, relative intestinal maturity can be a factor in mouse translational research. The murine intestine during the 14 d of life is far more permeable than that of a term human neonate and only later develops into a more translationally comparable organ.[36] This difference potentially could inform the interpretation of data regarding the timing of antibiotic administration to human and mouse neonates. Additional research in mice has shown that exposure prior to birth has a stronger effect on an obese phenotype than exposure after weaning.[25] Because macaques mature faster than humans, timing of administration could have been a factor in the findings for our current study, if the susceptible window for effect on growth rate for macaques differs appreciably from the 6-mo time point chosen.

Human studies have shown that antibiotics administration at an early age has a greater growth promotion effect on male infants than female.[30,37] Similar sex-associated differences have been found in mice.[5] Because most male macaques are removed prior to the onset of puberty, sex asymmetry in breeding groups of rhesus macaques becomes standard by 5 y of age. Selection criteria designed to follow an animal through early adulthood in an outdoor setting therefore led to the inclusion of almost 10 times more female than male macaques in our current study, although the statistical methods used to assess the data dismiss a sex-associated bias. This situation is due to the social structure of macaque groups, which, as is common for harem breeders, contain relatively few postpubescent males. Before the onset of puberty, which happens around age 3 y in macaques,[14] the sex ratio in a group is roughly 50:50. In the wild, male macaques are expelled from their natal groups around this time; therefore in standard husbandry practices, most postpubertal males are removed from the breeding group, given that leaving them in can create a perceived threat to the dominance of the breeder male and can endanger both the life and health of the younger male and the stability of the group as a whole. In addition, the removal of postpubertal male macaques is desirable to maintain genetic heterogeneity and prevent inbreeding.[14] The outdoor corrals at our center contain as many as 100 to 150 adult females but typically only 3 to 10 adult male macaques.

Some human studies have shown that male infants who received antibiotics at an early age experience a greater growth-promoting effect than female babies.[1,3,30,37] Similar sex-associated differences arose in some mouse studies, although others did not find this same sex-associated bias or did not control for sex.[7,10] Similarly, some human studies showed growth increases in both males and females given antibiotics;[5,37] some human studies[28,39] did not control for infant sex but showed the same antibiotic-related effect. Many showed the growth promotion effect to be correlated with male sex, infants given multiple courses, and those who received macrolide antibiotics, with lesser effects noted in females, those administered single courses, and those given antibiotics other than macrolides.[5,37,39] Although many studies found the effects to be persistent, one study found that the changes resolved over time (between 38 mo and 7 y of age).[39] One

study found that antibiotic administration occurred both earlier and at a higher rate in boys than girls, and these differences may help explain the outcomes that find a higher incidence of effects in boys.[37]

We evaluated weight as a function of the growth of our rhesus macaques, as is standard practice in mouse studies addressing this topic. In contrast, many human studies report BMI, although some also report growth rate (the measure we used here). Interestingly, one recent human study that tracked rate of weight change (as did our current study), rather than BMI, over the first 7 y of life and found no clinically significant differences between children exposed to antibiotics and those who were not.[15] Although BMI is the factor typically used to define overweight and obesity for the human population, this parameter is not commonly monitored in either mouse or NHP medicine because height is not commonly evaluated and is less applicable than in humans. BMI is used in some macaque models of obesity and potentially could be adapted for use in this research. Alternatively, body condition scores could be used to approximate BMI in NHP, although the ideal translational prospective project would mimic the human data and proactively obtain height measurements of study subjects. Reviewing the body condition scores of NHP would provide information regarding an animal's tendency toward leanness or overconditioning but was not possible for our population. Body condition scoring was validated in 2012 to correlate to percentage body fat in adult (not infant or juvenile) rhesus macaques;[8,38] the timing of this validation necessarily reduces the population for which this parameter would be documented in health records, because macaques born in 2012 had not yet reached the 5-y time point at the time of submission. However, body condition scoring is now included as a standard element of the annual exam for all animals at the Oregon National Primate Research Center, thus facilitating the use of this marker in modern populations.

Potential follow-up research regarding this topic is wide ranging. An evaluation of specific antibiotic types and durations, especially the differences between broad- and narrow-spectrum antibiotics with a focus on macrolides, seems particularly interesting. Comparing a younger subset of animals to account for maturity differences between human and rhesus infants in a group of animals that could be weighed more frequently would provide additional information. An evaluation of the types of health problems encountered that require antibiotic treatment may be informative, and, although respiratory disease and otitis are relatively uncommon in NHP, a comparison of trauma with diarrhea may highlight the effect of concurrent gastrointestinal disease states on any potential effect of early antibiotics. Comparing body condition scores rather than weights would likely provide a better analogy to BMI, because animals with similar weights may have vastly different body fat percentages; however collecting the data required for BMI evaluation or using a dexascanner to directly evaluate body fat percentage would provide the best translation of results from NHP to humans. Evaluating additional male NHP may be illuminating. Assessing the effect of changing husbandry practices may provide insight, because although the current data set was of inadequate size to make this comparison, that these changes have the potential to affect growth seems logical. To obtain a larger dataset, a multicenter retrospective approach could be considered but would reduce the current level of variable control, because significant differences in environments and subtle differences in animal husbandry between centers might also affect growth and thus confound data interpretation.

In conclusion, routine antibiotic use in young NHP does not lead to the same type of altered growth patterns that occur in humans. Our results from rhesus macaques therefore differ from those of human and murine studies. Research in mice supports the idea that antibiotic type and timing of administration, animal sex, and microbiome makeup contribute to the effect on growth.[4,10,24] However, although broad similarities exist between the human and murine microflora, significant differences in diet, hygiene (especially the habit of coprophagy), and basic intestinal and immunologic anatomy leave mice an imperfect model. Furthermore, although the outcomes of mice and humans exposed to antibiotics in early life are similar, the mechanisms may be quite different.[16,33] The creation of an NHP model that facilitates the study and understanding of the mechanisms by which antibiotics lead to obesity in later life potentially could allow the elucidation of preventive measures for infants requiring antibiotics, reveal additional risk factors, and offer other advantages over current murine models. Increased understanding of the reasons for the differences between NHP, humans, and mice with regard to the effects of early antibiotic administration on growth rate may provide insights into how best to treat early childhood illnesses without inadvertently increasing risk of obesity later in life.

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