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Antibiotics detected in urines and adipogenesis in school children

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ABSTRACT

Background: Although antibiotic use during early life has been demonstrated to be related to the altered adipogenesis in later life, limited data are available for the effect of antibiotic exposure in school children on adiposity from various sources, including from the use or contaminated food or drinking water.
Objective: To explore the association between the internal exposure of antibiotics from various sources and adipogenesis in school children using the biomonitoring of urinary antibiotics.
Methods: After 586 school children aged 8–11 years were selected from Shanghai in 2013, total urinary concentrations (free and conjugated) of 21 common antibiotics from six categories (macrolides, β-lactams, tetracyclines, fluoroquinolones, sulfonamides, and phenicols), including five human antibiotics (HAs), two antibiotics preferred as HA, four veterinary antibiotics (VAs), and ten antibiotics preferred as VA, were measured by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Creatinine-corrected urinary concentrations of antibiotics were used to assess their exposure. Overweight or obesity was determined by the body mass index or waist circumference-based criteria deriving from national data.
Results: All 21 antibiotics were found in urines with the overall detection frequency of 79.6%. The multinomial logistic regression analyses showed the significant associations of overweight and obesity with the exposure to VAs and antibiotics preferred as VA, but not with HAs or antibiotics preferred as HA. After adjusted for a number of obesity-relevant variables, the odds ratios (95% confidence interval) of BMI-based obesity risk of tertiles 2 and 3 of urinary concentrations relative to tertile 1 were respectively 2.54 (1.27, 5.07) and 2.92 (1.45, 5.87) for florfenicol, 0.57 (0.12, 2.63) and 3.63 (1.41, 9.32) for trimethoprim, and 3.00 (1.56, 5.76) and 1.99 (0.99, 4.01) for sum of veterinary antibiotics. Similar results were found when the outcome used WC-based obesity risk. The associations were sex related and mainly observed in boys.
Conclusions: Some types of antibiotic exposure, which were mainly from food or drinking water, were associated with an increased risk of obesity in school children. Due to the cross-sectional design, more longitudinal and experimental studies are warranted to further test these findings.

1. Introduction

Since it was discovered that chlortetracycline could improve animal growth in 1940s, a variety of antibiotics have been demonstrated to have growth promoting effects at a sub therapeutic dose level (Castanon, 2007; Dibner and Richards, 2005). The recognized mechanism of growth promoting is through the disruption of gut microbiota, which is found to be closely connected with the metabolism and immunity in body (Lin, 2011; Vijay-Kumar et al., 2010). Given that antibiotics are also extensively used in human, the possibility of similar effects of antibiotic exposure in children is arising and gaining more concern (Riley et al., 2013). A few well-designed animal experiments found the effects of antibiotic exposure early in life on gut microbiota, metabolism, and adipogenesis of mice at an exposure level of mg/kg/day and that the effects could last later in life (Cho et al., 2012; Cox et al., 2014). Several epidemiological studies also observed that the antibiotic use during early life was positively associated with childhood obesity risk (Ajslev et al., 2011; Azad et al., 2014; Bailey et al., 2014; Murphy et al., 2014; Trasande et al., 2013). Because human gut microbiota generally becomes stable like adults by three years old (Yatsunenko et al., 2012), these findings collectively suggested that the antibiotic use before the establishment of gut microbiota was one risk factor of childhood obesity (Cox and Blaser, 2014). However, the effect of antibiotic exposure after the establishment of gut microbiota on adipogenesis in human remains unknown.

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As a consequence of extensive antibiotic use in animal and human, various antibiotics have been detected in food and water environment (Bu et al., 2013; Leung et al., 2013; Liu and Wong, 2013; Riley et al., 2013). This suggests that the antibiotic exposure might be simultaneously from their use and contaminated food or water environment, but because the exposure measurement methods are based on questionnaire survey or prescription examination, current epidemiological studies inevitably ignore antibiotic exposures from contaminated food and water environment and focus on antibiotic use (Ajslev et al., 2011; Azad et al., 2014; Bailey et al., 2014; Murphy et al., 2014; Trasande et al., 2013). The biomonitoring approach of antibiotic exposure can overcome this problem by measuring the internal exposure dose related to various exposure sources (Wang et al., 2014). Using the biomonitoring approach of antibiotics in urine, several studies showed that children were extensively exposed to antibiotics not only form their use at a high-dose level of mg/kg, but also from contaminated food or drinking environment at a low-dose level of μg/kg (Ji et al., 2010a, 2010b, 2010c; Wang et al., 2015). Given that antibiotic use is usually a short-term event while exposure to antibiotics from contaminated food or drinking water can be continuous, children were inferred to have two different exposure modes: one is the short-term high-dose exposure to antibiotics by clinical utilization or self-medication and another is the long-term low-dose exposure to antibiotics from contaminated food or drinking water. To our best knowledge, however, there have been no specific studies so far, which investigated the associations of the antibiotic exposure from contaminated food or drinking water with the adipogenesis in children.

In this study, using the biomonitoring approach of urinary antibiotics of more than 500 school children living in Shanghai, we examined the association between the internal exposure of common antibiotics from various sources, including their use or contaminated food or drinking water, and the adipogenesis in children after the establishment of gut microbiota.

2. Material and methods

2.1. Study population

Due to a dense population and intensive breeding industry, people living in Shanghai is expected to have a high antibiotic consumption, as well as a high antibiotic environmental exposure (Zhang et al., 2015) and so children in this city were selected as target population. To better represent children in Shanghai, in cooperation with local district Centers for Disease Control and Prevention, two study sites were respectively established in the downtown and suburb of the city in 2013. After two preliminary schools were respectively selected from two study sites, three or four classes were randomly selected from each of third, fourth, and fifth grades in each school. After a small fraction of children more than 12 years old, of minority, or lack of data on age, sex, or ethnicity were excluded, a total of 586 Han children aged 8–11 years entered this study. Among them, all provided first morning urine, but 81 students did not participated in questionnaire survey and four students did not participated in routine physical examination. Finally, there were 505 students who completed all the study components. Of them, 213 came from the urban school and 292 came from the suburban school; 261 were boys and 244 were girls; and 240 aged 8–9 years and 265 aged 10–11 years. The study was reviewed and approved by the Institutional Review Board of Fudan University.

2.2. Collection of urines and anthropometric measurement

First morning was used in this study and urine collection was performed by participants themselves. After being handed to technicians in morning, urine samples were immediately aliquoted, transported to the lab in an ice chest as soon as possible, and frozen at −80 °C in the dark until analysis. Anthropometric measurement was performed by trained technicians following standard procedure. Body weight was measured to the nearest 0.1 kg and standing height (cm) and waist circumference (WC) were measured to the nearest 0.1 cm.

2.3. Antibiotic exposure assessment

To better represent antibiotic exposure, besides 18 antibiotics detected in urines in our previous study (Wang et al., 2015), other three common phenicols were added. So a total of 21 antibiotics were selected from six categories, including five macrolides, two β-lactams, three tetracyclines, four fluoquinolones, four sulfonamides, and three phenicols, were selected as target antibiotics (Table 1). Among them, five are only used as human antibiotics (HA), four are only used as veterinary antibiotics (VA), two are preferred as HA, and ten are preferred as VA. Using a modified method from that established previously in our lab (Wang et al., 2014), total urinary concentrations (free and conjugated) of 21 antibiotics were measured by the isotope dilution two-dimensional ultra-performance liquid chromatography coupled to high-resolution quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF MS). The creatinine-corrected urinary concentrations of antibiotics were used to assess their exposure.

The analytical method of urinary creatinine and 18 of 21 antibiotics, except for three phenicols, were previously provided in detail (Wang et al., 2015). For three phenicols, the analytical method was briefly described as followed: after an aliquot (1.0 mL) of urine was spiked with isotope-labelled chloramphenicol and hydrolyzed by β-glucuronidase, the mixture was purified by solid-phase extraction (SPE) and analyzed by two-dimensional UPLC-Q/TOF MS in negative ion mode with a mobile phase of 0.2% ammonium hydroxide water solution to retain antibiotics on trapping column and a mobile phase of acetonitrile and water to separate antibiotics. During entire analytical process, the interbatch recoveries of three phenicols varied between 86.1% and 114.3% with the interbatch relative standard deviations ranging from 3.8% to 11.6% and those of rest 18 antibiotics varied between 75.7% and 122.6% with the interbatch relative standard deviations ranging from 7.3% to 19.7%. Details of analytical method and quality control for phenicols were provided in Supplementary Information.

2.4. Statistical analysis

Body mass index, an indicator of overall adiposity, was calculated from the weight in kilograms divided by height in meters squared. Subjects were categorized into three groups of normal weight, overweight, and obesity according to BMI-based age- and sex-specific criterion proposed by the Working Group on Obesity in China (WGOC) (Ji, 2005). WC is used as a proxy measure of central body fat, which is thought as a better predictor for metabolic syndrome than the overall body fat. So subjects were also divided into three groups of normal weight, overweight, and obesity according to the age- and sex-specific cutoff values of the 85th and 95th percentiles of WC data from 2008 Chinese National Surveillance on Students Physical Fitness and Health and a study in the Hong Kong Special Administrative Region China (Ji et al., 2010a, 2010b, 2010c); the 85th percentile was selected based on the capability of predicting cardiovascular risk factors (Yan et al., 2008).

Twenty-one antibiotics were grouped by their antibacterial mechanism or usage and new variables were generated by a direct sum of mass concentration (ng/mL) of antibiotics in urine. Four new variables by the usage were HAs, VAs, antibiotics preferred as HA, and antibiotics preferred as VA. Six new variables by antibacterial mechanism were macrolides, β-lactams, tetracyclines, fluoquinolones, sulfonamides, and phenicols. Descriptive analysis provided the detection frequencies or selected concentration percentiles of antibiotics and new variables in relation to BMI categories of normal weight, overweight, or obesity. The rank sum test was used to analyze the differences of BMI, WC, and antibiotics with sex, study sites, age, or other demographic variables,