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Antibiotic exposure in infancy and development of BMI and body composition in childhood

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ABSTRACT

Background: It has been hypothesized that antibiotic usage in early life could contribute to development of overweight in childhood. Studies have seen association between antibiotic usage and overweight in childhood. We aimed to investigate the relationship between antibiotic exposure in infancy and development of body mass index (BMI) and body composition.

Methods: A prospective mother–child cohort study of 738 pregnant women and their 700 children, Copenhagen Prospective Studies on Asthma in Childhood$_{2010}$ (COPSAC$_{2010}$). Information on antibiotic exposure was collected by interviews. Height/length and weight measures were collected at age 1, 2, 3, 4, 5 and 6 years and body composition was determined by a dual-energy X-ray absorptiometry (DXA) scan at age 3.5 and 6 years.

Findings: 306 (46%) of the 661 children were exposed to antibiotics before 1 year of age. There were no differences in BMI z-score development at age 1–6 years between children exposed to antibiotics compared to unexposed: z-score difference, −0.06 (95%CI: −0.17;0.06), $p = 0.33$, and no sex-differences (p-interaction = 0.48). Children exposed vs. not exposed to antibiotics had comparable fat percentage at 6 years of age: log(mean difference), 0.60% (95%CI: −0.212 to 1.41), $p = 0.15$.

Interpretation: Children exposed to antibiotics had similar BMI, BMI z-score and body composition between 1 and 6 years of life compared to unexposed children. Our study does not support the hypothesis that antibiotic exposure in infancy leads to development of obesity in the first 6 years of life.

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1. Introduction

The prevalence of overweight and obesity in children and adolescents has been increasing worldwide [1–3], but seems to have reached a plateau in the recent years in Denmark [4]. This increase in prevalence of overweight and obesity has happened over a relatively short period and cannot be explained by genetic predisposition and changing dietary habits alone [5]; hence, some of the explanation could be found in other environmental exposures [6].

Antibiotics for treatment of infections are widely used in children during their first years of life [7]. It has been hypothesized that antibiotic usage in early life could be one of the environmental exposures contributing to development of overweight in childhood [8]. In support of this theory, it is known that antibiotics can be used as growth promoters in livestock, however, the growth promoting effect is poorly understood [8]. It is known that antibiotics can alter the gut microbiome [9,10] and recent research has suggested that manipulation of the gut microbiota and it's metabolic pathways can affect host's adiposity and metabolism [11], thereby linking the use of antibiotics with a potential for weight gain.

Some studies in children have reported that antibiotics in the early life was associated with a higher body mass index (BMI) later in childhood, but some of these studies were based on questionnaires, parent-reported antibiotic intake and outpatient prescriptions, with the potential risk of bias and confounding [8,12–14].

The aim of this study was to analyze the association between antibiotic exposure before 1 year of age and development of BMI, BMI z-

Abbreviations: BMI, body mass index; COPSAC$_{2010}$, COpenhagen Prospective Studies on Asthma in Childhood$_{2010}$; DXA, dual-energy X-ray absorptiometry; IOTF, International Obesity Task Force; TBLH, total body less head

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Research in context

Evidence before this study:

Antibiotics for treatment of bacterial infections are widely used in children. Early usage of antibiotic has been suggested as a cause of overweight and obesity in childhood, but findings are ambiguous.

We searched PubMed for clinical trials and meta analyses on this subject published in English, between 2014 until 2016, using the search terms "Antibiotic usage", "overweight/obesity", "growth" and "infant/child". We searched the reference lists of the retrieved articles. We found no meta-analysis on the topic.

We found that the earlier studies were predominantly based on questionnaires, parent-reported antibiotic intake and outpatient prescriptions, with the potential risk of bias and confounding. Additionally, they had only few or no information on relevant confounders.

Added value of this study:

This study does not support earlier reports that use of antibiotics within the first year of life leads to increased BMI or adiposity later in childhood. There is no consistent evidence supporting the role of clinical use of antibiotics in later development of obesity.

Implications of all the available evidence:

There is not convincing evidence supporting the role of early life antibiotics in development of obesity to change prescription practice for this purpose. Furture studies including randomized controlled trials should be performed to further explore this question.

score and body composition from 1 to 6 years in the Danish population-based prospective mother–child cohort Copenhagen Prospective Studies on Asthma in Childhood$_{2010}$ (COPSAC$_{2010}$).

2. Methods

2.1. Study population

COPSAC$_{2010}$ is an ongoing Danish mother–child cohort study of 738 unselected pregnant women and their 700 children. They are followed prospectively from pregnancy week 24 with 13 subsequent scheduled clinic visits at pregnancy week 36, 1 week, 1, 3, 6, 12, 18, 24, 30, 36, 48, 60 and 72 months, together with acute care visits [15,16]. In addition, parents kept a structured diary with daily registrations of symptoms and medicine consumption from birth.

Inclusion criteria were available anthropometrics data (weight and length/height) at least at 1 and/or 6 years of age combined with completed medical interviews (detailed information about antibiotic usage), and a daily diary that was at least 90% fulfilled and validated (detailed information about number of disease episodes). Children not fulfilling these criteria were excluded.

The daily diary cards were filled from birth by parents monitoring symptoms of illness and registering daycare absenteeism due to illness. The diary cards were reviewed with the family by the COPSAC pediatricians at each visit to validate symptom definitions. For the first 3 years of life the children were seen in the research clinic at age 1 week, 1 month, 3 months, 6 months and six-monthly hereafter. Furthermore, we correlated the daily dairy card information with the prescribed medication for the children.

2.2. Endpoints

2.2.1. Anthropometrics

Height/length and weight were measured without clothes by trained research assistants. We used calibrated digital weight scales. Length was measured until the age of 2 years with Kiddimetre, Raven Equipment Ltd [17]. Height at later ages was measured with a stadiometer: Harpenden. Holtain Ltd., Crymych, Dyfed, Wales, which was calibrated yearly. BMI was calculated by weight in kilograms divided by height squared in meters using BMI at 1, 2, 3, 4, 5 and 6 years as outcomes. International Obesity Task Force (IOTF) cut-offs for BMI were used to determine risk of overweight and obesity (above grade zero) [18].

2.2.2. Dual-energy X-ray absorptiometry (DXA) scans

DXA scans were performed at 3.5 and 6 years of age as a whole-body scan with Lunar iDXA, GE Healthcare, Fairfield, CT, USA. The data from the DXA scans were examined and validated following recognized guidelines by experts in nuclear medicine, who were blind to the study exposure and had no involvement in the following data analyses [19]. DXA analyses were performed with enCoreTM software, GE Healthcare Lunar © 2011, version 14.10.022. We used fat percentage in the compartment total body less head (TBLH) as outcome adjusted for sex and age at measurement [20]. Pediatric measurement precision was not determined on-site. The acquisition and analysis software provides a standard deviation for total body fat percentage of 0.4%. The acquisition and analysis software supports pediatric whole-body scans. We did not calculate Z-scores based on a reference population as we reported the absolute measurements and based our analyses on these values. All DXA scans were performed on the same scanner. Previous publications on the precision of fat percentage estimation using the Lunar iDXA scanner found coefficients of variation (CV) between 0.9% and 1.0% by repeat scans on the same day [21,22]. Even though this was found in an adult population, and to our knowledge has not been studied in children of a similar age-group to our study population, we have no reason to believe that the CV in our study would be significantly different, at least at the age of 6 years. Consequently, although variability could result in type 2 statistical error we do not find this likely due to the very low CV. Regarding measurement error (systematic bias) this would not influence the results due to the cross-sectional study-design.

2.3. Antibiotic exposure

Detailed information on antibiotic exposure was obtained during interviews with the participants at the scheduled clinical visits and validated against the structured daily diary cards and The Danish National Prescription Registry, which includes records on all drugs collected at the pharmacy [23]. Antibiotic usage in this study was registered according to WHO's ATC classification system and only systemic administered antibiotics were used as determinants in our analyses. Antibiotic usage was analyzed as a dichotomous variable (yes/no) before the age of 1 year. In addition, we used antibiotic courses as a continuous variable and in a subanalysis we stratified the number of antibiotic courses into: 0, 1, 2, >2 to investigate any potential cumulative effect of antibiotic exposure. We also divided antibiotics into a group of narrow-spectrum antibiotics (penicillins and ampicillin derivates) and a group of antibiotics which can be considered as more broad-spectrum antibiotics (macrolides, beta-lactams with beta-lactamase inhibitor, cephalosporins, dicloxacillins, aminoglycosides, trimethoprims and sulphonamides).

2.4. Covariates

Information regarding sex, race, maternal age at birth, gestational age in weeks, mode of delivery, older children in the home, furred animals in the home, social circumstances, passive smoking, smoking during pregnancy, gestational diabetes, antibiotics during pregnancy, antibiotics at birth administered to the mother, maternal asthma status and length of exclusive breastfeeding were gathered during the clinic visits and validated against register data whenever possible. Length of exclusively breastfeeding was calculated in days. When the child's diet was supplemented or replaced by continually use (>7 days) of infant formula or other foods, we considered exclusive breastfeeding as terminated.

Information on maternal pre-pregnancy weight was collected from the pregnancy records and maternal BMI was calculated using the height measured at the COPSAC clinic.

Social circumstances were defined by the z-score of the first component of a principal component analysis (PCA) on household income, maternal age and maternal level of education at offspring age 2 years (explained 55% of the variance) [24].

Birth length and weight were obtained at the first clinic visit one week after birth by personal interview and validated against the Danish Medical Birth Registry. Birth weight for gestational age z-score was derived from ultrasound based intrauterine growth curves [25].

Number of disease episodes (cold, tonsillitis, otitis media, pneumonia, fever, pseudocroup and/or gastrointestinal infection) were obtained by the daily diary recordings, requiring that each episode had to be separated with at least one day without any symptoms.

2.5. Statistical analyses

We analyzed the association between antibiotic exposure and the cross-sectional assessments of BMI at age 6 years and body composition at age 3.5 and 6 years using generalized linear models adjusted for all potential confounders associated with antibiotic exposure in the first year of life ($p<0.10$). Estimates are presented as differences in BMI and fat percentage for children exposed to antibiotics compared to their unexposed peers among the entire study group and stratified for sex.

The main analysis of BMI was BMI z-score tracking over time analyzed by a similarly adjusted mixed model taking account of repeated measures, using WHO sex specific BMI z-scores obtained at every scheduled visit from 1 to 6 years [26]. Z-scores were used since BMI does not have a linear development [27]. This model included only data from 1 to 6 years, since the exposure variable was de termined from birth to 1 year of age.

Log-transformation was performed for fat percentage values to achieve normality of the residuals prior to analysis; log transformation was chosen by a box cox transformation.

The mothers and children in this cohort participated in a single-center, double-blind, placebo-controlled, parallel-group study with fish oil and high dose vitamin D supplementation in third trimester of pregnancy [16,28]. There were no interaction between the interventions and antibiotic exposure in first year of life (data not shown).

Normality of data distribution was evaluated by histograms and a significance level of 5% was used in all analyses. Analyses were performed in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

2.6. Ethics

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the Local Ethics Committee (H-B-2008-093), and the Danish Data Protection Agency (2015-41-3696). Both parents gave written informed consent before enrolment. Data validation and quality control followed the guidelines for good clinical practice. Data was collected during visits to the clinical research unit and stored in a dedicated online database.

3. Results

3.1. Baseline characteristics

700 children were included, of which 28 were excluded due to pre-term birth and 11 due to incomplete records on antibiotic exposure, leaving 661 eligible children for the current study. 306 (46%) of the 661 included children were exposed to treatment with systemic antibiotics before age 1 year. 168 (25%) of the 306 children were treated once, 65 (10%) twice and 74 (11%) children were treated three times or more. Of the prescribed courses, 512 (88%) were beta-lactams (ampicillin derivates, penicillins, beta-lactams with beta-lactamase inhibitor or dicloxacillins), 66 (11%) of the courses were macrolides, aminoglycosides or cephalosporins, and 6 (1%) of the courses were of other types.

Table 1 shows baseline characteristics of children exposed to antibiotics and their unexposed peers. Older children in the home, higher maternal pre-pregnancy BMI, antibiotic usage during pregnancy, passive smoking, and disease episodes in the first year of life were all significantly associated with exposure to antibiotics in the first year of life ($p<0.05$) and were subsequently included in the final models as potential confounders. Children exposed to antibiotics had lower social circumstances. This was borderline significant ($p<0.07$) and was also included in as a potential confounder. Furthermore, we included exclusive breastfeeding duration as a confounder, because of its known influence on growth in early childhood [29]. As expected, the number of disease episodes in the first year of life was significantly higher among children exposed vs. unexposed to antibiotics: mean no. episodes (SD), 8.2 (3.9) vs. 7.2 (4.0), p-value < 0.01.

3.2. Antibiotic exposure and BMI z-score development during childhood

The development of BMI z-score from age 1 year until age 6 years in children exposed vs. unexposed to systemic antibiotics before age 1 year is illustrated in Fig. 1, showing no differences between the groups, except at 2 years of age where the BMI z-score estimate is lower for the exposed vs. not exposed children, this was the same after adjustment for confounders (data not shown). In line with this, the repeated measurement mixed model adjusted for the confounders mentioned above, showed no difference in BMI z-score from 1 year to 6 years of age between children exposed vs. not exposed to antibiotics: −0.06 (95% CI: −0.17; 0.06), p-value = 0.33. Sex-stratified repeated measurements models also showed no difference in BMI z-score between children exposed to antibiotic and their unexposed peers: girls, −0.04 (95% CI: −0.21; 0.13), p-value = 0.62, and boys, −0.08 (95% CI: −0.24; 0.08), p-value = 0.38, p-interaction = 0.48.

A post-hoc analysis of the 80 children, who were exposed to antibiotic before 6 months of age, revealed no difference in BMI z-score development until to age 6 years between the exposed and unexposed peers (data not shown).

3.3. Number of antibiotic courses, type of antibiotics, BMI and BMI z-score development

We subsequently examined a possible dose-response relationship between number of antibiotic courses in the first year of life and BMI z-score development. A repeated measurement analysis using number of antibiotic courses (0 ($N=355$), 1 ($N=168$), 2 ($N=65$), >2 ($N=74$)) as a categorical exposure variable showed no differences in BMI z-score from 1 year to 6 year of age: −0.02 (95% CI: −0.08; 0.04), p-value = 0.58. Furthermore, we analyzed the subpopulation of children whom had broad spectrum antibiotics during the first year of life. We found no difference between the children exposed to broad

Table 1
Baseline characteristics of the $COPSAC_{2010}$ cohort according to antibiotic exposure in the first year of life.

	Missing data	Full cohort	Exposed to antibiotic before 1 year of age	Unexposed to antibiotic before 1 year of age	*P*-value
Participants, N (%)	–	700 (100%)	306 (46%)	355 (54%)	–
Females (yes), N (%)	–	340 (49%)	141 (46%)	182 (51%)	0.18†
Caucasian (yes), N (%)	–	669 (96%)	295 (96%)	339 (96%)	0.55†
Birth weight for gestational age z-score, units, mean (SD)	–	0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.46‡
Gestational age in weeks (without preterm), mean (SD)	–	40.1 (1.2)	40.0 (1.2)	40.1 (1.3)	0.37‡
Age, mean (SD)					
- 1 year data, +/- 3 months (days)	15	368.4 (15.8)	367.7 (15.0)	368.8 (16.0)	0.35‡
- 6 years data, +/- 6 months (years)	54	6.0 (0.2)	6.0 (0.2)	6.0 (0.2)	0.93‡
Delivery by cesarean section (yes), N (%)	–	151 (22%)	65 (21%)	68 (19%)	0.51†
Older children in the home (yes), N (%)	10	395 (57%)	199 (65%)	186 (53%)	**<0.01**†
Furred animals at home (yes at 1 years visit),* N (%)	14	338 (49%)	144 (48%)	182 (52%)	0.30†
Disease episodes in first year of life, mean (SD)	49	7.6 (3.9)	8.2 (3.9)	7.2 (4.0)	**<0.01**‡
Exclusive breastfeeding duration, mean (SD)	8	103.1 (60.0)	102.7 (61.8)	104.5 (57.3)	0.71‡
Maternal pre-pregnancy BMI, mean (SD)	84	24.5 (4.3)	25.1 (4.7)	24.1 (4.0)	**<0.01**‡
Social circumstances (PCA), mean (SD)	–	0 (1.0)	−0.08 (1.0)	0.06 (1.0)	0.07‡
Passive smoking in first year of life (yes), N (%)	11	375 (54%)	180 (59%)	177 (50%)	**0.02**†
Maternal smoking during pregnancy (yes), N (%)	–	54 (8%)	22 (7%)	27 (8%)	0.84†
Gestational diabetes (yes), N (%)	3	16 (2%)	8 (2%)	8 (3%)	0.76†
Antibiotics during pregnancy (yes), N (%)	–	253 (36%)	126 (41%)	114 (32%)	**0.02**†
Maternal antibiotics at birth (yes), N (%)	–	223 (32%)	92 (30%)	106 (30%)	0.89†
Maternal asthma (yes), N (%)	4	187 (27%)	87 (29%)	86 (24%)	0.18†
Maternal age in years at birth, mean (SD)	–	32.3 (4.4)	32.1 (4.5)	32.4 (4.3)	0.45‡

* Yes = minimum of 30 days (per year) with furred animal(s).
† Chi-squared test.
‡ Student's T-test.

spectrum antibiotics in the first year of life vs. the unexposed children: −0.07 (95% CI: −0.19; 0.05), *p*-value = 0.26. A cross-sectional analysis of BMI at age 6 years showed that children exposed to antibiotics did not have a higher BMI compared to the unexposed group in the unadjusted nor adjusted analysis: adjusted mean difference, 0.07 kg/m^2 (95% CI: −0.16; 0.31), *p*-value = 0.55. In our dose−response analysis we did not find any significant cumulative effect of antibiotic. In our subanalysis we saw that children exposed to two courses had a significantly higher BMI than the unexposed group: 0.44 kg/m^2 (95% CI: 0.01; 0.89) *p*-value = 0.04 in unadjusted analysis, however only trend significant after confounder adjustment: 0.36 kg/m^2 (95% CI: −0.04; 0.75) *p*-value = 0.07. There was no association for children exposed to three courses or more (data not shown). We saw no difference in the BMI at 6 years of age between those children who had broad spectrum antibiotics in the first year of life and the children who were not exposed to antibiotics (data not shown).

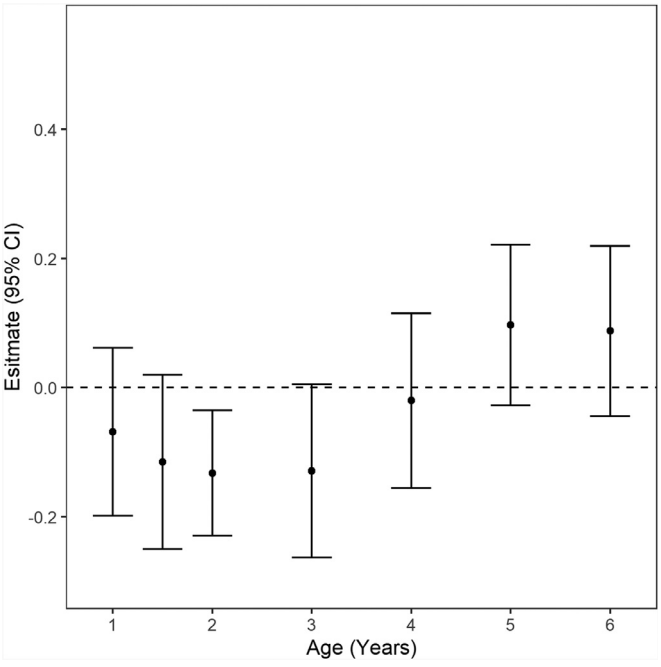

Fig. 1. Development of BMI z-score from age 1 year until age 6 years in children exposed vs. unexposed to systemic antibiotics before age 1 year. Associations between antibiotic exposure before 1 year of age and BMI z-score through childhood illustrated by mean difference in BMI z-score for exposed children compared to children unexposed at each visit and 95% confidence intervals.

3.4. Antibiotic exposure and fat percentage

The mean age for the completion of the two DXA scans was 3.5 years (SD: 0.34) and 6.2 years (SD: 0.24), where 467 (70.1%) and 500 (75.6%) children had usable scan data, respectively. Children exposed to antibiotics did not show any differences in fat percentage at either 3.5 or 6 years of age compared to children not exposed to antibiotics: 3.5 years, difference 0.85% (95% CI: −1.84 to 3.55), *p*-value = 0.53, and 6 years, difference 0.60% (95% CI: −0.212 to 1.41), *p*-value = 0.15 (Table 2). We did not find any differences in our dose-response analysis either (data not shown).

4. Discussion

This study showed no association between usage of antibiotics in the first year of life and BMI z-score development until 6 years of age. Furthermore, there was no difference in fat percentage determined by DXA scans at age 3.5 or 6 years of age, which argues against a role of early life antibiotic exposure on development of childhood obesity.

The present study uses data from the prospectively monitored clinical mother−child cohort study $COPSAC_{2010}$, where participants

Table 2
Association between antibiotic usage and fat percentage from DXA scans.

A: $3^1/_2$ years of age, B: 6 years of age.				
	Crude		Adjusted*	
	Exposed Geometric Mean (SD)	Unexposed Geometric Mean (SD)	Estimate (95% CI)**	*P*-value
A: Fat%				
On total body less head	28.3 (4.4) ($n = 216$)	28.4 (4.5) ($n = 251$)	0.85 (−1.84 to 3.55)	0.53
B: Fat%				
On total body less head	24.3 (1.2) ($n = 221$)	23.6 (1.2) ($n = 279$)	0.60(−0.21 to 1.41)	0.15

* Adjusted for: age, sex, disease episodes 1. year of life, passive smoking 1. year of life, maternal BMI, antibiotic during pregnancy, other child in house, birth weight for gestational age, exclusive breastfeeding and social circumstances.
** Logged values of fat percentage.

were followed closely by trained research nurses and paediatricians. A significant strength of the study is the repeated anthropometric assessments collected at a single research unit with subsequent rigorous data validation, where all data was double-checked against source data.

Another advantage is the data on fat percentage measured by DXA scans at two age-points, which is a reliable method for quantifying body composition in children. It was performed by experienced laboratory technicians, and all scan data was validated by experts in nuclear medicine [17,30,31].

Additionally, our study utilizes very reliable, prospectively collected data on antibiotic usage in early life, which was validated against the structured daily diary cards and The National Prescription Registry. By this procedure, we possibly have minimized recall bias and excluded antibiotics from the analysis, which were prescribed but never filled, and which were collected at the pharmacy, but never ingested.

We had information on a broad range of potential cofounders, which were collected at personal interviews with the families and validated against register data. This allowed for robust adjustments for potential confounders such as sex, child weight for gestational age, pre-pregnancy BMI, antibiotics usage during pregnancy, social circumstances and passive smoking [32,33]. Furthermore, we had unique data on number of disease episodes captured in daily diary cards with closed response categories (yes/no) filled out by the parents and was reflective of use of antibiotics, the use of diary cards were well validated in our earlier cohort [34]. To our knowledge this measure for the load of infectious diseases and antibiotic exposure in early childhood is unparalleled.

One limitation of this study is the relative low number of participants, compared to previous questionnaire based studies [8,35]. This particularly resulted in a loss of statistical power in our dose−response and sex stratified analyses. Another limitation is the lacking information on weight gain in the mothers during pregnancy and hence we could not adjust our analyses for this potential confounder.

We chose to analyze differences in BMI estimates and not overweight or obesity, since we only had 31 children in risk of overweight or obesity at age 6 years estimated by the International Obesity Task Force (IOTF) grades [18].

Several studies have reported associations between antibiotic exposure in infancy and growth measures in childhood, but most of these were based on questionnaires or health care records with large differences in antibiotic information, age of anthropometric measurements, and confounder adjustment, and the overall results have been ambiguous [8,13,35−38]. Also animals studies have demonstrated that antibiotics have the ability to perturb the microbiota especially in infancy leading to long-term health consequences [39,40]. In humans; one study reported an association between antibiotic exposure in the first 6 months of life and increased BMI at age 10−38 months, but they did not find a significant association of exposure to antibiotics at <6 months with child overweight or obesity at 7 years [8]. Another study, however, reported an association between antibiotic exposure before 6 months of age and risk of overweight at 7 years of age, but only among children born by normal weight mothers, whereas an opposite association was observed among children born by overweight mothers [37]. Two other studies showed association between antibiotic exposure in infancy and risk of overweight or increased BMI, but only in boys [14,38]. Two recent studies proposed that the association with increased BMI was mainly found after broad-spectrum antibiotic treatment: The first study showed a correlation between antibiotic exposure before 6 months of age and BMI at age 2 years, which was more pronounced if the prescribed antibiotic was a macrolide [12]. The second study showed that having ≥4 courses of antibiotics increased the risk of obesity at 2−5 years of age, this effect was present if the prescribed antibiotics were broad-spectrum, which accounted for more than 20% of the courses, but there were no significant association if the prescribed antibiotics were narrow-spectrum [35]. In our dose-response analysis we do not see any cumulative effect of antibiotics. We saw that 2 courses were borderline significant for a higher BMI, but for three courses or more there was no association with BMI, so we interpret this as a spurious finding.

It is known that there is a higher use of both antibiotics in general and especially of broad-spectrum antibiotics in particular in the United States compared to Denmark [41,42]. Murphy et al. observed that children exposed to antibiotics within the first year of life ranged from 22% to 76% across 18 countries. Only three other countries had a lower prevalence of exposure to antibiotics compared to our cohort from Denmark [14]. This is reflected in the low prevalence of broad-spectrum antibiotic use of 11% (macrolides, aminoglycosides and cephalosporins) in our cohort. In a sub-analysis comparing the children who had broad spectrum antibiotics in the first ear of life with those without antibiotics we did not see a difference in BMI between 1 and 6 years of age. Our data do not support the hypothesis that broad spectrum antibiotic in early life increases BMI later in life.

Recently, two studies reported negative findings in line with ours: One study showed that children exposed to antibiotics in their first 6 months of life did not have a different growth in early childhood compared to those not exposed [43]. Similarly, another study did not find any impact of early antibiotic usage and changes in fat percentage at 3.5, 7 or 11 years of age [44].

There are several possible explanations for the observed differences. Most studies lack to our belief sufficient confounder adjustment. We found that antibiotic use was associated with older children in the home, maternal pre-pregnancy BMI, passive smoking, disease episodes in the first year of life, antibiotic use during pregnancy, and social circumstances. This could imply that antibiotic use early in life is a proxy for socio-economic status, and thereby some of the previous observations could be explained by the relationship between differences in socio-economics and childhood growth [45,46]. A very important known confounder is maternal BMI that is associated with overweight in the offspring [33] and this confounder is lacking in

many studies [12,14,35,44]. Our results are strengthened by the identification of several possible confounders, which have not been incorporated in previous reports. This strengthens the conclusions drawn from our results.

Our study, conducted in a prospective cohort does not support that exposure to antibiotics in early life increases BMI or fat percentage later in childhood. Future studies should focus on subpopulations receiving broad-spectrum antibiotics or repetitive doses of antibiotics in infancy and include the burden of disease episodes as a covariate. Furthermore, the hypothesis that antibiotic exposure in infancy causes persistent changes in the children's gut microbiome should be further explored.

This study does not support earlier reports that use of antibiotics within the first year of life leads to increased BMI or adiposity later in childhood. There is no consistent evidence supporting the role of antibiotics in later development of obesity. Therefore, the authors suggest that the hypothesis of antibiotic usage in infancy as a causative factor for later obesity should not be taken into consideration, when deciding whether to treat with antibiotics or not in a clinical setting.

Contributors' statement page

Drs. Sejersen and Vinding drafted the initial manuscript, and reviewed and revised the manuscript.

Prof. Bisgaard is the guarantor of the study, from conception and design to conduct of the study and acquisition of data and contributed considerably to the analyses, interpretation of the data and also reviewed and revised the manuscript.

Drs. Stokholm, Chawes, Krakauer and Bønnelykke have provided important intellectual input and contributed considerably to the analyses, interpretation of the data and also reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Governance

We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH) good clinical practice guidelines and the Helsinki Declaration. We follow national and international rules on the processing of personal data, including the Danish Act on Processing of Personal Data and the practice of the Danish Data Inspectorate.

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Declaration of Competing Interest

The authors have nothing to disclose.

Financial disclosure

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2019.10.020.

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