International Journal of Obesity (2015) **39,** 16–25

www.nature.com/ijo

PEDIATRIC ORIGINAL ARTICLE

The intestinal microbiota composition and weight development in children: the KOALA Birth Cohort Study

LEJM Scheepers[1], J Penders[1,2], CA Mbakwa[1], C Thijs[1], M Mommers[1] and ICW Arts[1,3]

OBJECTIVE: To investigate whether the intestinal microbiota composition in early infancy is associated with subsequent weight development in children.

METHODS: Analyses were conducted within the KOALA Birth Cohort Study ($n = 2834$). This cohort originates from two recruitments groups: pregnant women with a conventional lifestyle (no selection based on lifestyle) and pregnant women recruited through alternative channels (organic shops, anthroposophic clinicians/midwives, Steiner schools and relevant magazines). From 909 one-month-old infants, fecal samples were collected and analyzed by quantitative PCR targeting bifidobacteria, *Bacteroides fragilis* group, *Clostridium difficile*, *Escherichia coli*, Lactobacilli and total bacteria counts. Between the ages of 1 and 10 years, parent-reported weight and height was collected at 7 time points. Age- and gender-standardized body mass index (BMI) *z*-scores were calculated. Data were analyzed using generalized estimating equation.

RESULTS: Colonization with *B. fragilis* group was borderline significantly associated with a higher BMI *z*-score of 0.15 (95% confidence interval (CI): − 0.02 to 0.31), in the conventional subcohort. After stratification for fiber intake ($P_{forinteraction} = 0.003$), colonization with *B. fragilis* group was associated with a 0.34 higher BMI *z*-score among children with a low-fiber intake in this subcohort (95% CI: 0.17–0.53). Higher counts among colonized children were positively associated with BMI *z*-score only in children within the conventional subcohort and a high-fiber diet (BMI *z*-score 0.08; 95% CI: 0.01–0.14), but inversely associated in children with a low-fiber diet (BMI *z*-score − 0.05; 95% CI: − 0.10 to 0.00), and in children recruited through alternative channels (BMI *z*-score − 0.10; 95% CI: − 0.17 to − 0.03). The other bacteria were not associated with BMI *z*-scores, regardless of subcohort.

CONCLUSION: Using a targeted approach, we conclude that the intestinal microbiota, particularly the *B. fragilis* group, is associated with childhood weight development. To identify the potential impact of additional bacterial taxa, further prospective studies applying an unconstrained in-depth characterization of the microbiota are needed.

International Journal of Obesity (2015) **39,** 16–25; doi:10.1038/ijo.2014.178

INTRODUCTION

Overweight and obesity can have serious health consequences, including type 2 diabetes and cardiovascular diseases that are major public health problems.[1] Not only in adults, but also in children, the prevalence of overweight and obesity is increasing.[2,3] The development of obesity is a complex process involving both genetic and environmental factors, such as an increased energy intake and reduced energy expenditure. However, these factors do not fully explain the increased obesity prevalence.[4,5]

Our human gut is colonized with a complex 100 trillion microbe cells, the intestinal microbiota. The intestinal microbiota composition varies between individuals; the development of the infant microbiota is mainly influenced by prenatal exposure: mode of delivery, type of infant feeding and antibiotic use.[6] Recently, the intestinal microbiota has been identified as a potential determinant of obesity, both in animal and human studies. The study by Ley *et al.*,[7] published in 2005, showed for the first time that the intestinal microbiota differs between lean and obese mice. Obese mice harbor significantly less Bacteroidetes and more Firmicutes compared with their lean siblings.[7] Furthermore, colonization of germ-free mice with an intestinal microbiota of obese donor mice led to a significantly greater increase in fat deposition than colonization with an intestinal microbiota of lean donors.[8] A limited number of human studies, usually comparing obese with normal-weight subjects in a cross-sectional design, have been conducted so far. In addition, the many different methods for characterization of the indigenous microbiota complicate the direct comparison of results between studies (summarized in Table 1). So far, studies have provided contradictory results: some demonstrated a reduced level of Bacteroidetes to be associated with obesity,[9–14] whereas others found the opposite[15–17] or no association.[18–22] Specific bacterial and archeal genera or species have also been associated with obesity in humans, such as the *Lactobacillus* spp,[10,22,23] bifidobacteria,[15,24,25] *Escherichia coli*[25] and *Methanobrevibacter smithii*[10,15,23] (Table 1), but findings were not consistent between studies. To determine whether a different microbiota composition in early infancy is related to subsequent weight development, longitudinal cohort studies are needed. To our knowledge, only four human observational studies investigated the relation between intestinal microbiota composition and weight development in a longitudinal manner,[25–29] two of which addressed microbiota in infancy with short-term follow-up but not beyond infancy[28,29] and two others addressed

[1]Department of Epidemiology, Maastricht University, CAPHRI School for Public Health and Primary Care, Maastricht, The Netherlands; [2]Department of Medical Microbiology, Maastricht University Medical Centre, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht, The Netherlands and [3]Department of Epidemiology, Maastricht University, CARIM School for Cardiovascular Diseases, Maastricht, The Netherlands. Correspondence: LEJM Scheepers, Department of Epidemiology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands.
E-mail: lieke.scheepers@maastrichtuniversity.nl
Received 31 January 2014; revised 4 September 2014; accepted 24 September 2014; accepted article preview online 9 October 2014; advance online publication, 11 November 2014

Table 1. Human studies on the intestinal microbiota in relation to body weight or weight development

Authors	*Participants*	*Study design*	*Method and community measured*	*Major findings*
White *et al.*[29]	218 Infants	Observational: prospective birth cohort study	BLAST *Enterococcus* spp., *Lactobacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Lachnospiraceae* spp., *Pseudomonas* spp., *Escherichia coli*, Gammaproteobacteria, *Varibaculum* spp., *Bifidobacterium*, *B. fragilis* group, *Bacteroides* spp.	*Bacteroides* spp. at day 30 was associated with reducing growth, expressed as weight (standardized for age and sex), in males at 6 months of age. *Saphylococcus* spp and *Escherichia coli*. at day 4 and detection from day 4 through to 30 was associated with expected growth in males as well as in females.
Bervoets *et al.*[22]	27 Normal-weight and 26 overweight/obese children (6–16 years)	Observational: case–control study	Quantitative culturing, MALDI-TOF MS, and qPCR *B. fragilis* group, *Bifidobacterium*, *Clostridium*, *Staphylococcus*, *Lactobacillus, Bacteroides-Prevotella-Porphyromonas* spp., *Bifidobacterium* spp., *Clostridium coccoides/Eubacterium rectale* group, *Clostridium leptum* group, *Staphylococcus* spp. and *Lactobacillus* spp.	Higher *B. vulgatus* (member of the *B. fragilis* group) concentrations in normal-weight vs overweight/obese children and adolescents. Higher *Lactobacillus* spp. concentrations in obese/overweight vs normal-weight children and adolescents. The Firmicutes/Bacteroidetes ratio was in favor of the Firmicutes in O/O children and adolescents.
Millon *et al.*[23]	47 Normal-weight and 68 obese adults	Observational: case–control study	qPCR Bacteroidetes, *Bifidobacterium animalis*, Firmicutes, *Lactobacillus* genus, *L. lactis* and *M. smithii*	Increased *L. reutri* and decreased *B. animalis*, *L. paracasei, L. plantarum* and *M. smithii* concentrations in obese vs normal-weight adults.
Karlsson *et al.*[21]	20 Normal-weight and 20 overweight children (4–5 years)	Observational: case–control study	qPCR *Lactobacillus*, *Bifidobacterium*, *Enterobacteriaceae*, *Akkermansia muciniphila*-like bacteria, *Desulfovibrio* and *B. fragilis* group	Higher *Enterobacteriaceae* and lower *Desulfovibrio* and *A. muciphilia*-like bacteria in overweight/obese vs normal-weight children.
Xu *et al.*[14]	91 Normal-weight, 62 overweight and 22 obese children (7–13 years)	Observational: case–control study	qPCR Bacteroidetes, Firmicutes and Bacteroidetes/ Firmicutes ratio	Higher Bacteroidetes concentrations in normal-weight vs obese children. Higher Bacteroidetes/ Firmicutes ratio in normal-weight vs overweight and obese children.
Greenblum *et al.*[59]	82 Normal-weight/ overweight and 42 obese participants	Observational: case–control study	Metagenomics, the whole microbiome	Differences in the microbiome (genes) from lean/overweight vs obese participants.
Ismail *et al.*[16]	28 Normal-weight (17 children and 11 adults) and 51 obese (23 children and 28 adults)	Observational: case–control study	qPCR *Bacteroidetes* and Firmicutes	Higher Firmicutes and Bacteroidetes concentrations in obese vs normal-weight people.
Vael *et al.*[28]	138 Normal-weight and obese preschool children	Observational: prospective birth cohort study	Culture *B. fragilis* group, *Bifidobacterium*, *Clostridium*, *Enterobacteriaceae*, *Enterococcus*, *Lactobacillus, Staphylococcus*	Higher *B. fragilis* group concentrations at 3 and 26 weeks of age were associated with increased BMI during follow-up at age 12, 18, 24, 30 and 36 months/preschool age. *Staphylococcus* at 3 and 52 weeks of age was associated with lower BMI *z*-scores in preschool children.
Zuo *et al.*[12]	52 Normal-weight and 52 obese adults	Observational: case–control study	Culture *B. fragilis* group, *Bifidobacterium*, *Clostridium*, *Enterobacteriaceae*, *Enterococcus*, *Lactobacillus*, *Staphylococcus Clostridium perfringens E. coli*, Enterococci and Lactobacilli	Lower *C. perfringens* and *Bacteroides* concentrations in obese vs normal-weight adults.
Luoto *et al.*[27]	15 Normal-weight and 15 overweight children (10 years of age)	Observational: nested case–cohort study	FISH *Bacteroides–Prevotella* group, *Bifidobacterium*, *Clostridium histolyticum* group, and *Lactobacillus*, *Lactococcus*, *Enterococcus*	Higher bifidobacteria concentrations (at 3 months of age) in normal-weight vs overweight children at 10 years of age (not sig.).
Santacruz *et al.*[60]	34 Normal-weight and 16 overweight pregnant women	Observational: case–control study	qPCR *B. fragilis* group, *Bifidobacterium*, *Lactobacillus* (*L. casei*), *Clostridium coccoides* group, *Enterobacteriaceae*, *E. coli*, *Staphylococcus* (*S. aureus*) and *Akkermansia muciniphila*	Increased *B. fragilis* group and *Bifidobacterium* and decreased *Enterobacteriaceae*, *E. coli* and *Staphylococcusin* concentrations in normal-weight vs overweight women. Increased *E. coli* concentrations in women with excessive weight gain vs women with normal weight gain over pregnancy. Increased *Bifidobacterium* in women with normal weight gain vs women with excessive weight gain during pregnancy.
Scwiertz *et al.*[15]	30 Normal-weight, 35 overweight and 33 obese adults	Observational: case–control study	qPCR *Actinobacteria* (*Bifidobacterium*), Bacteroidetes (*Bacteroides* and *Prevotella*), Firmicutes (*C. leptum* group, *C. coccoides* group, *E. cylindroides* group, Lactobacilli/Enterococci, *Ruminococcus flavefaciens* subgroup, *Veillonella*) and *Archea* (*Methanobrevibacter* genus)	Higher Bacteroidetes concentrations in overweight participants vs normal-weight adults. Low *Bifidobacterium* and *C. leptum* concentrations in overweight vs normal-weight adults. The ratio of Firmicutes to Bacteroidetes changed in favor of the Bacteroidetes in overweight and obese adults.
Santacruz *et al.*[25]	36 Overweight and obese adolescents (13–15 years)	Intervention study: weight loss program		Increased *B. fragilis* group and *Lactobacillus* group and decreased *C. coccoides* group, *Bifidobacterium (longum* and

Table. 1. (Continued)

Authors	*Participants*	*Study design*	*Method and community measured*	*Major findings*
			qPCR *B. fragilis* group, *C. coccoides*, *C. leptum*, *Lactobacillus*, *E. coli* and *Bifidobacterium* (*longum*, *breve*, *bifidum*, *adolescentis*, *catenulatum*)	*adolescentis*) concentrations after losing weight. The *B. fragilis* group, *C. Leptum*, *Bifidobacterium catenulatum* concentrations were higher whereas concentration of *C. coccoides* group, *Lactobacillus* group and *Bifidobacterium* (*breve* and *bifidum*) were lower in the high-weight-loss group than in the low-weight-loss group before and after the intervention. Increases with excessive weight loss.
Balamurugan *et al.*[18]	13 Normal-weight and 15 obese children (11–14 years)	Observational: case–control study	qPCR *Bacteroides–Prevotella*, *Bifidobacterium* species, *Lactobacillus acidophilus* group, *Eubacterium rectale* and *Faecalibacterium prausnitzii*	Higher *Faecalibacterium prausnitzii* concentrations in obese vs normal-weight children.
Mai *et al.*[20]	14 Normal-weight and 14 obese African and Caucasian adults (at least 40 years of age)	Observational: case–control study	FISH and qPCR *Bacteroides–Prevotella* group, *bifidobacteria*, *Clostridium coccoides–Eubacterium rectale* group and *Clostridium cluster XIVa* (*eubacteria*, *clostridia* and *ruminococci*)	No difference in microbiota composition between normal-weight and obese adults, regardless of detection method.
Armougom *et al.*[10]	20 Normal-weight, 20 obese and 9 anorexia nervosa adults	Observational: case–control study	qPCR Bacteroidetes, *Lactobacillus*, Firmicutes and *Methanobrevibacter smithii*	Higher *Bacteroidetes* and *Lactobacillus* concentrations in obese vs normal-weight and anorexic adults. High *M. smithii* concentrations in normal-weight vs anorexic adults.
Zhang *et al.*[18]	3 Normal weight and 3 obese (3 post gastric bypass)	Observational: case–control study	qPCR, most sequences belong to Firmicutes and Bacteroidetes. The others: *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia*	Higher *Prevotellaceae* and *Archaea* concentrations in obese vs normal-weight or post gastric bypass individuals.
Turnbaugh *et al.*[12]	31 Monozygotic and 23 dizygotic twin pairs (21–32 years old) and 46 mothers	Observational: cross-sectional study	16S rRNA, the microbiome	Low Bacteroidetes and high *Actinobacteria* concentrations in obese vs normal-weight adults. Obesity was associated with reduced bacterial diversity.
Nadal *et al.*[14]	39 Overweight and obese adolescents (13–16 years)	Intervention study: calorie-restricted diet and increased physical activity	FISH *Bacteroides/Prevotella*, *Bifidobacterium*, C. *histolyticum*, *C. lituseburense*, *E. rectale/C. coccoides*, *Escherichia coli*, *Enterobacteriaceae*, *L. bacillus/Enterococcus* Roseburia subcluster	Low *C. histolyticum* and *E. rectale/C. coccoides* concentrations associated with weight loss. High *Bacteroides/Prevotella* concentrations in adolescents who lost more than 4 kg of weight.
Kalliomäki *et al.*[27]	24 Normal-weight and 25 overweight or obese children (7 years)	Observational: prospective birth cohort, case–cohort study	FISH and qPCR *Bacteroides–Prevotella*, *Bifidobacterium* group, *Clostridium histolyticum* group, *Lactobacillus*, *Lactococcus*, *Enterococcus* and *Satphylococcus aureus*	High bifidobacteria and low *Satphylococcus aureus* concentrations (at 6 and 12 months) in normal-weight vs overweight and obese children.
Duncan *et al.*[20]	14 Normal-weight and 33 obese males	Observational: case–control study	FISH Bacteroidetes, *Bifidobacteri*a and the *Clostridium coccoides* including the *Eubacterium recatle* group and *Roseburia*	No difference in microbiota composition between normal-weight and obese males.
Collado *et al.*[24]	36 Normal-weight and 18 overweight pregnant women	Observational: case–cohort study	FCM-FISH and qPCR *B. fragilis* group, *Bifidobacterium* genus, *Clostridium histolyticum* group, *Lactobacillus–Enterococcus* group and total counts	High *B. fragilis* group and *Satphylococcus aureus* concentrations in overweight women vs normal-weight women. High *B. fragilis* group concentrations were associated with excessive weight gain over pregnancy.
Ley *et al.*[10]	2 Normal-weight and 12 obese people.	Observational: case–control study and intervention study	16S rRNA Bacteroidetes and Firmicutes	Low Bacteroidetes concentrations in obese vs normal-weight people. Bacteroidetes increases and Firmicutes concentrations decreases during weight loss over a period of 1 year. Bacteroidetes concentrations were correlated with percentage loss of body weight.

Abbreviations: BLAST, Basic Local Alignment Search Tool; BMI, body mass index; FCM-FISH, fluorescent *in situ* hybridization coupled with flow cytometry; FISH, fluorescence *in situ* hybridization; MALDI-TOF MS, matrix-assisted laser desorption/ ionization mass spectrometry; O/O, overweight/obese; qPCR: quantitative PCR; sig., significant.

microbiota at ages 6–12 months and 3 months, in children with follow-up until 7 years and 10 years, respectively[25–27] (Table 1).

Several mechanisms have been put forward to explain how the interaction between microbiota and host metabolism may contribute to obesity.[30,31] Certain species are able to digest dietary fiber, that is, complex carbohydrates that cannot be degraded by human enzymes, thus yielding energy for microbial growth and end products such as short-chain fatty acids. The latter have profound effects on human metabolism because they can serve as energy substrates for the gut (butyrate) and peripheral tissues (acetate, propionate), modulate inflammation and wound healing and act as vasodilators.[30,31] In addition, short-chain fatty acids can signal through G protein-coupled receptors, such as GPR41, on enteroendocrine cells, inducing the secretion of peptide YY.[32,33]

The aim of this study was to investigate whether the intestinal microbiota composition in early infancy is associated with subsequent weight development during childhood. The fecal samples of 909 one-month-old infants were analyzed with quantitative PCR assays to enumerate several bacterial groups and species and related to weight development up to the age of 10 years.

MATERIALS AND METHODS

Subjects and study design

The current analyses were conducted within the KOALA Birth Cohort Study in the Netherlands. The design of the KOALA study has been described in detail elsewhere.[34] Briefly, this cohort originates from two recruitment groups: healthy pregnant women with a conventional lifestyle ($n = 2343$) and pregnant women recruited through alternative channels ($n = 491$). The women with a conventional lifestyle were retrieved from an on-going prospective pregnancy cohort study on pregnancy-related pelvic girdle pain in the Netherlands.[35] The second group of pregnant women were recruited through alternative channels, that is, posters in organic food shops, anthroposophic doctors and midwives, anthroposophic under-five clinics, Rudolf Steiner schools and magazines for special interest groups. This latter group of women was considered to have an alternative lifestyle that could involve dietary habits (vegetarian, organic), child-rearing practices, vaccination schemes and/or use of antibiotics. All participants were enrolled at 34 weeks of gestation.

Women recruited from January 2002 until December 2002 ($n = 1176$) collected a fecal sample from their child ~1 month postpartum. Inclusion criteria for the present analyses were: availability of a fecal sample collected between 3 and 6 weeks of life, sufficient amount of feces (≥1 g) and parental completion of the accompanying questionnaire (fecal collection questionnaire).

Exclusion criteria were: prematurity (infants born before 37 weeks of gestation), twins, congenital abnormalities related to growth (such as Down's syndrome, Turner syndrome, Fallot's tetralogy multiple disabilities), administration of antimicrobial agents before feces collection and children without any body mass index (BMI) measurement (Figure 1). All parents signed the informed consent and the study was approved by the medical ethics committee of Maastricht University Medical Center+.

Fecal collection and microbial analysis

Parents were asked to collect the feces of their child at 1 month postpartum. They received a feces tube with a spoon attached to the lid (Sarstedt, Nümbrecht, Germany), together with a sanitary napkin, an instruction form about the correct collection and sending procedure and a brief questionnaire. Parents placed a sanitary napkin in the diaper to prevent absorption by the diaper; collection of the feces was done by spoon and deposited in the tube. The tube was sent to the Medical Microbiology department at Maastricht University Medical Center+ by post as soon as possible. Transport time was minimized by asking the parents to collect the feces on a Monday, Tuesday or Wednesday so that the samples did not remain in the mail over the weekend.

At the laboratory, fecal samples were 10-fold diluted in peptone water (Oxoid CM009, Hampshire, UK) containing 20% v/v glycerol (Merck, Darmstadt, Germany) and stored at − 20 °C until analysis.

DNA extraction from the feces and the subsequent microbial analyses by means of real-time PCR assays has been described in detail elsewhere.[6] Briefly, the DNA was extracted by a combination of bead-beating and the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). DNA from all fecal samples was subjected to real-time PCRs for quantification of bifidobacteria, *Bacteroides fragilis* group, *Clostridium difficile*, *E. coli*, Lactobacilli and total bacteria based on 16S rDNA gene sequences. For detection of the bifidobacteria, C. *difficile*, *E. coli* and members of the bacteroides, the 5′-nuclease technique was used. For quantification of Lactobacilli and total bacteria load, real-time detection of PCR products was conducted by means of SYBR Green I (Bio-Rad Laboratories, Hercules, CA, USA). The validation of the real-time PCR assays has been described in detail elsewhere.[36–38] Log_{10} colony-forming units per g (log_{10} CFU g^{-1}) were calculated for each stool sample from the threshold cycle values by using the constructed standard curves. The prevalence of colonization was expressed as the percentage of infants colonized with a specific bacterial group or species. As almost all infants were colonized with bifidobacteria, a cutoff value of 10.68 log_{10} CFU g^{-1} (the median) was used to divide the population into those with a low or high abundance of the genus.

Outcome variable

Information on the child's weight, height and age at the time of measurements was collected using self-administered questionnaires. Parents were asked to report their child's body weight and height at seven different time points. At the first and second time points, where the children had an average age ± s.d. of 11 ± 1 months and 22 ± 3 months, respectively, parents were asked to report the most recent weight and height measured at the child health clinic, and to also report the age (in months) at the time of these measurements. At the further follow-up time points, in 2006 (age 56 ± 4 months), 2007 (age 73 ± 5 months), 2008 (age 80 ± 5 months), 2009 (age 92 ± 5 months) and 2010 (age 103 ± 5 months), parents were asked to measure weight (in kg specified to one decimal) and height (in cm) without shoes and clothes and to report the exact date of measurement. BMI (weight/height^2, kg m^{-2}) was standardized by recoding it into age- and gender specific BMI *z*-scores using data from the Dutch reference population.[39]

Statistical analysis

The characteristics of the participants are given as mean values ± s.d. for continuous variables and as numbers and proportions for categorical variables. Missing values for continuous covariables were replaced with the mean (maternal prepregnancy BMI $n = 2$, age at collection of fecal sample $n = 10$), and missing values for categorical covariables were classified as 'unknown' (place and mode of delivery $n = 23$, maternal education of the mother $n = 10$).

Generalized estimating equation (GEE) models with unstructured correlation structure were used for analysis of the repeated BMI *z*-scores. The analyses included only one bacterial group or species at a time. When bacterial counts were used as an independent variable, only infants who were colonized with that specific bacterial group or species were included. The age of the child at the time of BMI measurement was included in all models as the time variable. We tested whether the association between intestinal microbiota composition and BMI *z*-scores differed with increasing age by including an interaction term in the GEE models. When the interaction term was significant ($P < 0.05$), we performed linear regression analyses for each BMI measurement separately. Participants were recruited through two different recruitment channels (representing a conventional or alternative lifestyle); we therefore tested for interaction between the intestinal microbiota and recruitment group. The interaction term was significant for *C. difficile* ($P = 0.037$) and *B. fragilis* group ($P = 0.032$); we therefore performed all analyses stratified for lifestyle.

First, unadjusted GEE analyses were performed to determine the association between colonization (yes/no) and bacterial counts (log_{10} CFU g^{-1}) and BMI *z*-scores. Second, adjusted GEE analyses were conducted that included potential confounders. If the potential confounder changed the regression coefficient of any of the main determinants by more than 10%, it was consequently included in all models. The following variables were considered: infant gender (male, female), place and mode of delivery (vaginal delivery at home, vaginal delivery in hospital, artificial delivery in hospital, cesarean section in hospital), birth weight (in g), maternal prepregnancy BMI (kg m^{-2}), age at collection of fecal sampling (in days), maternal smoking during pregnancy (number of cigarettes per day), type of infant feeding in the first month (exclusive breastfeeding, exclusive

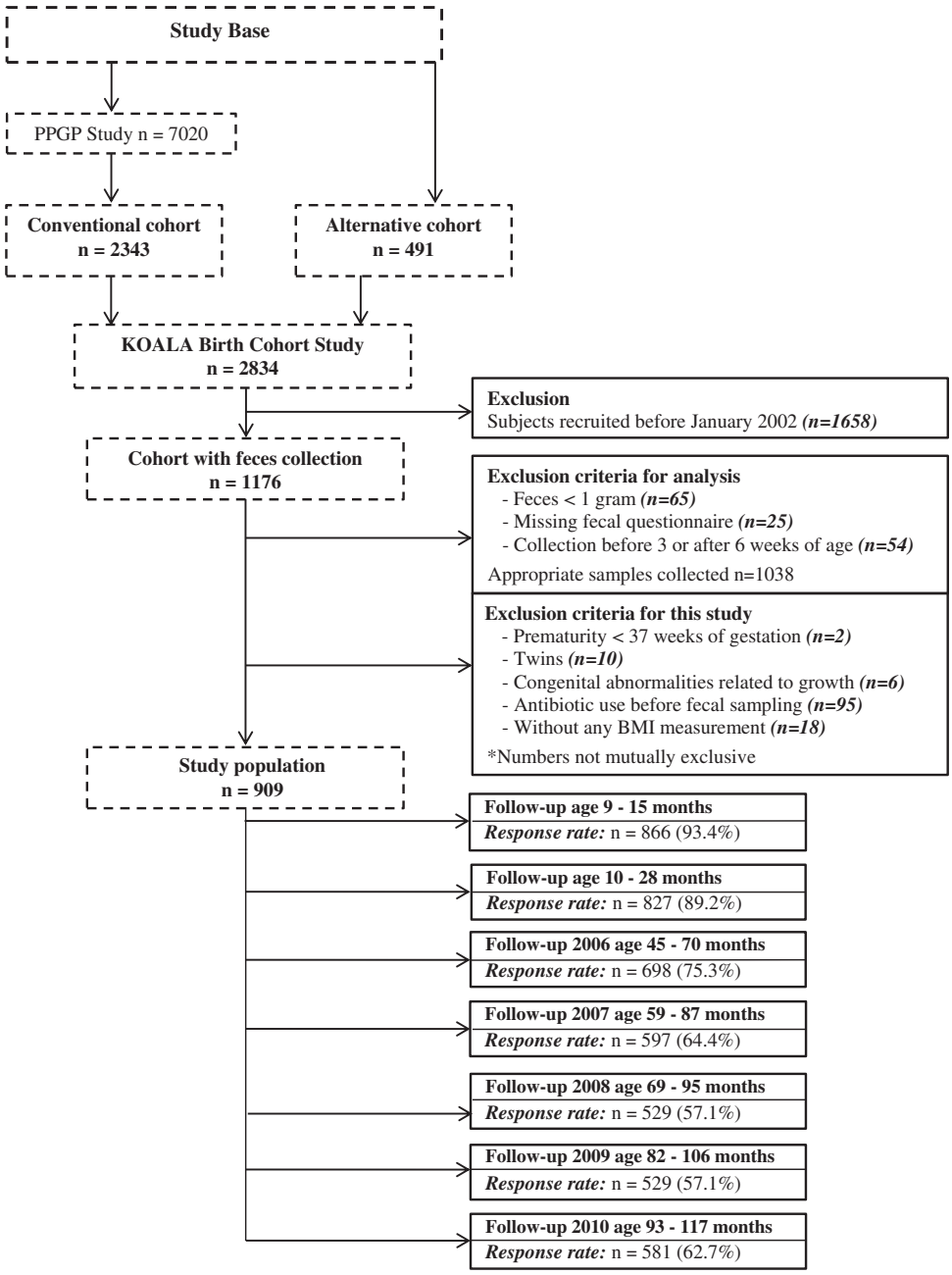

Figure 1. Flowchart of the study population. The KOALA Birth Cohort study included pregnant women with a conventional lifestyle (recruited from the on-going Pregnancy-related Pelvic Girdle Pain study (PPGP)) or an alternative lifestyle (recruited from 'alternative' channels). Participants recruited from January 2002 onwards were asked to collect a fecal sample of their child. Reasons for exclusion with numbers, and response rates on the seven weight and height questionnaires are presented.

bottle-feeding or a combination), duration of breastfeeding (months), education level of the mother (lower education, vocational education, higher general secondary/pre-university or higher vocational/academic education) and total counts of bacteria. Finally, a third model additionally included the following dietary variables: total energy intake, energy percentage from fat and energy percentage from protein. Dietary information was collected during a period of 4 weeks by a food frequency questionnaire (FFQ) administered at the age of 4 years. The FFQ was specifically developed to assess children's energy intake and validated by using the doubly labeled water method.[40] The questionnaire consisted of 71 items and, in addition, for 27 foods the specific types or brands consumed and preparation methods were asked. Parents reported their child's habitual food consumption by indicating the frequency of consumption ('never' to '6–7 days a week') and by specifying proportion sizes in natural units (for example, pieces, slices), household units (for example, glasses of spoons) or g (for example, g of fish). Parents were asked to measure volume of the cups and glasses they used for the children. The average energy intake (kJ) and fiber intake (in g per MJ) per day were calculated using the Netherlands Food Composition Table 2001 (NEVO).[41] For the products that were not included in the NEVO 2001 table, the nutritional values were provided by a dietician.

Fiber intake was considered as a potential effect modifier. Information on dietary fiber intake at the age of 4 years was also obtained from the FFQ. To determine whether dietary fiber modified the associations between the intestinal microbiota and weight development, a test for interaction with fiber intake was conducted in both recruitment groups. If the interaction term was significant, the analysis was stratified by level of dietary fiber intake (above or below the median of 15.0 g per day).

Data analysis was performed using the SPSS statistical software package version 19.0 (SPSS Inc., Chicago, IL, USA). The unadjusted (crudeβ) and

adjusted (adjβ) regression coefficients with the corresponding 95% confidence interval (95% CI) are presented. A *P*-value of < 0.05 was considered statistically significant in all analyses.

RESULTS

Of the 1176 collected fecal samples, the samples that were too small (< 1 g; $n = 65$), were collected before the age of 3 weeks or after the age of 6 weeks ($n = 54$) or where the fecal questionnaire was missing ($n = 25$) were excluded. In total, 1032 fecal samples were appropriate for microbial analysis.[6] After exclusion of premature infants ($n = 2$), twins ($n = 10$), children with congenital abnormalities related to growth ($n = 6$), children without any BMI measurement ($n = 18$) and children who received antibiotics before fecal sampling ($n = 95$), the study population consisted of 909 children (Figure 1). Table 2 shows the baseline characteristics of the KOALA cohort ($n = 2834$) and the study population ($n = 909$). In general, the two groups were comparable, except that the study population had more participants with an alternative lifestyle, a higher level of maternal education and longer duration of breastfeeding, but less mothers who smoked during pregnancy. These differences are mainly because of the period of fecal sampling that coincided with recruitment of the alternative group. Almost all infants were colonized with bifidobacteria (98.6%), followed by *E. coli* (88.2%) and *B. fragilis* group (81.2%), whereas colonization with *C. difficile* (25.3%) and Lactobacilli (31.9%) was less frequent (Table 2). Neonatal colonization with *B. fragilis* group in the conventional subcohort was associated with a statistically significant higher BMI *z*-score of 0.16, compared with infants who were not colonized (Table 3). However, this did not remain significant after adjustment (Adjβ 0.11; 95% CI: −0.05 to 0.26). Other bacterial groups or species were not associated with BMI *z*-scores in children in the conventional subcohort. In children from the alternative subcohort (Table 4), a higher count of *B. fragilis* group was associated with a significantly lower BMI *z*-score in both crude and adjusted models (Adjβ −0.07; 95% CI: −0.15 to 0.00). Other bacterial groups or species were not associated with BMI *z*-scores in the alternative subcohort.

In the conventional subcohort, the interaction term between colonization with *B. fragilis* group and fiber intake was significant ($P = 0.003$). There was no significant interaction with fiber for any of the other bacteria. After stratification for fiber intake (Table 5) in children with a lower than median fiber intake (< 15 g per day), colonization with *B. fragilis* group was associated with a higher BMI *z*-score (Adjβ 0.34; 95% CI: 0.17–0.53); however, higher counts of *B. fragilis* group in colonized children was associated with a lower BMI *z*-score after adjustment for potential confounders (Adjβ −0.05; 95% CI: −0.10 to 0.01). Furthermore, in children with a higher than median fiber intake (> 15 g per day), colonization with *B. fragilis* group was not associated with BMI *z*-scores, but a higher count of *B. fragilis* group was associated with a higher BMI *z*-score (Adjβ 0.07; 95% CI: 0.01–0.14). For all analyses, adding the dietary variables to the adjusted models (model 3) did not substantially alter the results.

Finally, we examined whether associations of bacterial groups and species with BMI *z*-scores changed over time (age of BMI measurement). Only for *C. difficile* the time interaction term was significant ($P = 0.002$). Linear regression analysis showed that children in the conventional subcohort, who were colonized with *C. difficile* at 1 month postpartum, had a lower BMI *z*-score of −0.24 (Adjβ 0.24; 95% CI: −0.45 to −0.03) at 103 ± 5 months of age. For the other time points, no significant effects were found (data not shown).

Table 2. Baseline characteristics of the KOALA Birth Cohort and the study population

	KOALA Birth Cohort Study	*Study population*
	(n = 2834)	(n = 909)
Determinants		
Prevalence of colonization with intestinal bacteria, *n* (%)		
Bifidobacteria	–	896 (98.6)
Escherichia coli	–	802 (88.2)
Clostridium difficile	–	230 (25.3)
Bacteroides fragilis group	–	738 (81.2)
Lactobacilli	–	290 (31.9)
Counts of intestinal bacteria ($\log_{10}$ CFU g^{-1}), median (range)[b]		
Bifidobacteria	–	10.69 (6.84–11.56)
Escherichia coli	–	9.30 (5.91–10.79)
Clostridium difficile	–	5.40 (2.70–9.57)
Bacteroides fragilis group	–	9.40 (5.74–10.36)
Lactobacilli	–	8.66 (7.92–10.73)
Total count	–	12.69 (8.47–19.63)
Covariables		
Age sampling feces (days), (mean ± s.d.)	–	31.7 ± 3.3
Recruitment group conventional	2343 (83%)	618 (68%)
Maternal education[a]		
Low	289 (10%)	60 (7%)
Middle	1060 (38%)	312 (34%)
High	1341 (48%)	508 (56%)
Other	108 (4%)	19 (2%)
Maternal prepregnancy BMI (kg m^{-2}), (mean ± s.d.)	23.6 ± 4.0	23.3 ± 3.6
Maternal smoking during late pregnancy		
Yes	200 (7%)	27 (3%)
Gender		
Boy	1451 (51%)	454 (50%)
Birth weight, g (mean ± s.d.)	3500 ± 512	3562 ± 467
< 2500	79 (3%)	12 (1%)
2500–4500	2663 (95%)	873 (96%)
> 4500	70 (3%)	24 (3%)
Place and mode of delivery		
Natural delivery at home	1187 (42%)	432 (48%)
Natural delivery in hospital	924 (33%)	301 (33%)
Artificial delivery at home[c]		2 (0%)
Artificial delivery in hospital[c]	223 (8%)	66 (7%)
Cesarean section in hospital	311 (11%)	93 (10%)
Duration of breastfeeding, months (mean ± s.d.)	4.7 ± 3.0	5.9 ± 4.5
Type of infant feeding		
Formula feeding from birth	446 (16%)	113 (12%)
Combination of breast and formula feeding in first 3 months	1185 (42%)	320 (35%)
Breastfeeding as the only milk feeding in first 3 months	614 (22%)	241 (26%)
Breastfeeding as the only milk feeding in first 6 months	562 (20%)	235 (26%)
Dietary factors		
Total energy intake, kcal (mean ± s.d.)	1466 ± 305	1445 ± 298
Energy percentage from fat, % (mean ± s.d.)	14.6 ± 2.1	14.4 ± 2.1
Energy percentage from protein, % (mean ± s.d.)	29.6 ± 4.2	29.4 ± 4.3
Energy percentage from carbohydrates, % (mean ± s.d.)	55.8 ± 5.0	56.1 ± 5.1
Fiber, g (mean ± s.d.)	15.3 ± 4.0	15.4 ± 4.1

Abbreviations: BMI, body mass index; CFU, colony-forming unit. Numbers do not always add up to the total because of missing data. [a]Low: primary school, preparatory vocational or lower general secondary school, middle: vocational, higher general secondary or pre-university education, high: higher vocational or academic education. [b]Includes only children who were colonized with the specific bacteria group or species. [c]Forceps or vacuum extraction.

DISCUSSION

The current study showed that colonization with *B. fragilis* group at 1 month postpartum tended to be associated with a higher BMI in children up to 10 years of age, in particular among children in the conventional subcohort and a low-fiber diet. In line with our results, Bäckhed *et al.*[42] showed that host total body fat content increased after colonization of germ-free mice with

Table 3. Generalized estimating equation (GEE) results for colonization and counts (log_{10} CFU g^{-1}) of the gut microbiota at 1 month of age and BMI *z*-score measured repeatedly between 1 and 10 years of age; includes only children with a conventional lifestyle ($n = 629$; 68% from the total study population)

		Crudeβ (95% CI)	P-*value*	*Adjβ (95% CI)*[a]	*P-value*	*Adjβ (95% CI)*[b]	P-*value*
Prevalence of colonization with intestinal bacteria, n *(%)*							
Bifidobacteria[c]	317 (49%)	0.11 (−0.01 to 0.22)	0.07	0.05 (−0.06 to 0.16)	0.38	0.05 (−0.07 to 0.16)	0.43
Escherichia coli	551 (89%)	−0.04 (−0.23 to 0.15)	0.68	−0.03 (−0.21 to 0.14)	0.73	−0.06 (−0.25 to 0.14)	0.57
Clostridium difficile	154 (25%)	−0.05 (−0.19 to 0.08)	0.44	−0.12 (−0.25 to 0.01)	0.08	−0.09 (−0.23 to 0.04)	0.18
B. fragilis group	511 (83%)	0.16 (0.00 to 0.32)	0.049	0.11 (−0.05 to 0.26)	0.17	0.15 (−0.02 to 0.31)	0.08
Lactobacilli	201 (33%)	−0.06 (−0.18 to 0.06)	0.34	0.06 (−0.17 to 0.06)	0.31	−0.08 (−0.02 to 0.05)	0.22
Counts of intestinal bacteria (log_{10} CFU g^{-1}), median (range)[d]							
Bifidobacteria	10.71 (6.84 to 11.56)	0.03 (−0.04 to 0.10)	0.38	0.00 (−0.06 to 0.07)	0.94	0.00 (−0.07 to 0.07)	0.96
Escherichia coli	9.43 (5.91 to 10.79)	−0.00 (−0.05 to 0.04)	0.93	−0.01 (−0.05 to 0.04)	0.78	0.01 (−0.04 to 0.05)	0.76
Clostridium difficile	5.24 (2.70 to 9.57)	−0.05 (−0.10 to 0.01)	0.13	−0.03 (−0.09 to 0.03)	0.26	−0.04 (−0.10 to 0.02)	0.19
B. fragilis group	9.40 (5.74 to 10.36)	0.01 (−0.04 to 0.05)	0.76	0.02 (−0.02 to 0.07)	0.29	0.01 (−0.03 to 0.06)	0.57
Lactobacilli	8.70 (7.92 to 10.73)	−0.02 (−0.16 to 0.13)	0.83	0.01 (−0.14 to 0.15)	0.94	−0.02 (−0.18 to 0.14)	0.81
Total count	12.55 (8.47 to 19.63)	0.05 (−0.04 to 0.15)	0.29	0.05 (−0.04 to 0.14)	0.28	0.02 (−0.07 to 0.12)	0.66

Abbreviations: Adjβ, adjusted β; BMI, body mass index; CFU, colony-forming unit; CI, confidence interval. Numbers in the table may not add up to 629 because of missing values. [a]Adjusted for age BMI measurements, infant gender, birth weight, place and mode of delivery, maternal education level, maternal BMI before pregnancy, duration of breastfeeding, age at collection of fecal sample, type of infant feeding and total bacteria counts. [b]Additionally adjusted for nutrition intake at 4 years of age (total energy intake (kcal), energy from fat (%) and energy from protein (%). [c]Colonization with bifidobacteria in association with standardized BMI presented as low (< 10.68 log_{10} CFU g^{-1}) versus high (> 10.68 log_{10} CFU g^{-1}) as almost all infants were colonized; uncolonized ($n = 13$) were added to the low count group. [d]Includes only children who were colonized with the specific bacteria group or species.

Table 4. Generalized estimating equation (GEE) results for colonization and counts (log_{10} CFU g^{-1}) of the gut microbiota at 1 month of age and BMI *z*-score measured repeatedly between 1 and 10 years of age; includes only children with an alternative lifestyle ($n = 298$; 32% from the total study population)

		Crudeβ (95% CI)	P-*value*	*Adjβ (95% CI)*[a]	*P-value*	*Adjβ (95% CI)*[b]	P-*value*
Prevalence of colonization with intestinal bacteria, n *(%)*							
Bifidobacteria[c]	142 (49%)	−0.04 (−0.21 to 0.12)	0.62	−0.08 (−0.24 to 0.08)	0.34	−0.13 (−0.29 to 0.04)	0.14
Escherichia coli	251 (86%)	0.09 (−0.12 to 0.30)	0.40	−0.13 (−0.33 to 0.08)	0.22	−0.17 (−0.38 to 0.03)	0.10
Clostridium difficile	76 (26%)	0.01 (−0.17 to 0.20)	0.88	−0.04 (−0.22 to 0.13)	0.63	−0.06 (−0.24 to 0.12)	0.52
B. fragilis group	227 (78%)	−0.05 (−0.25 to 0.14)	0.60	−0.01 (−0.18 to 0.16)	0.89	0.01 (−0.16 to 0.18)	0.94
Lactobacilli	89 (31%)	0.00 (−0.18 to 0.19)	1.00	−0.06 (−0.22 to 0.11)	0.51	−0.04 (−0.21 to 0.13)	0.62
Counts of intestinal bacteria (log_{10} CFU g^{-1}), median (range)[d]							
Bifidobacteria	10.66 (6.85 to 11.49)	0.00 (−0.80 to 0.09)	0.93	−0.03 (−0.11 to 0.04)	0.40	−0.05 (−0.13 to 0.02)	0.17
Escherichia coli	9.30 (5.92 to 10.62)	−0.02 (−0.08 to 0.04)	0.57	−0.01 (−0.07 to 0.05)	0.70	−0.02 (−0.09 to 0.04)	0.48
Clostridium difficile	5.78 (2.85 to 8.81)	−0.01 (−0.07 to 0.06)	0.88	0.04 (−0.03 to 0.11)	0.24	0.07 (−0.00 to 0.14)	0.04
B. fragilis group	9.18 (5.80 to 10.33)	−0.07 (−0.14 to 0.00)	0.05	−0.07 (−0.15 to 0.00)	0.05	−0.10 (−0.17 to −0.03)	0.01
Lactobacilli	8.61 (7.95 to 10.33)	−0.06 (−0.26 to 0.14)	0.57	−0.12 (−0.33 to 0.10)	0.29	−0.06 (−0.25 to 0.13)	0.55
Total count	12.85 (9.13 to 19.04)	0.05 (−0.04 to 0.15)	0.29	0.05 (−0.04 to 0.14)	0.28	0.02 (−0.07 to 0.12)	0.66

Abbreviations: Adjβ, adjusted β; BMI, body mass index; CFU, colony-forming unit; CI, confidence interval. Numbers in the table may not add up to 298 because of missing values. [a]Adjusted for age BMI measurements, infant gender, birth weight, place and mode of delivery, maternal education level, maternal BMI before pregnancy, duration of breastfeeding, age at collection of fecal sample, type of infant feeding and total bacteria counts. [b]Additionally adjusted for nutrition intake at 4 years of age (total energy intake (kcal), energy from fat (%) and energy from protein (%). [c]Colonization with bifidobacteria in association with standardized BMI presented as low (< 10.68 log_{10} CFU g^{-1}) versus high (> 10.68 log_{10} CFU g^{-1}) as almost all infants were colonized; uncolonized ($n = 13$) were added to the low count group. [d]Includes only children who were colonized with the specific bacteria group or species.

Bacteroides thetaiotaomicron, the most abundant member of the *B. fragilis* group in the human gut. These latter results were explained by the ability of *B. thetaiotaomicron* to ferment plant- or host-derived polysaccharides to short-chain fatty acids, in particular acetate. Acetate can serve as a source of energy for peripheral tissues, and is taken up by the liver and used as a substrate for lipogenesis and gluconeogenesis. Colonized mice have higher levels of liver triglycerides that suppress the expression of fasting-induced adipose factor and increase the storage of triglycerides in adipocytes.[30,31] Our results are furthermore in accordance with the observational study by Vael *et al.*,[28] who showed a positive association between *B. fragilis* group in the feces of 3- and 26-week-old infants, and BMI *z*-score at preschool age (up to 36 months) ($n = 138$). In contrast to our study, White *et al.*[29] showed that presence of *Bacteroides* spp. in 30-day-old infants was associated with lower body weight *z*-scores at the age of 6 months in males ($n = 108$), but not in females. However, these studies used traditional culture and a microarray respectively to assess the microbiota composition, and caution is therefore required when comparing these results with our study. The role of fiber in children colonized with members of the *B. fragilis* group is contradictory. Only in children with a low-fiber diet (< 15 g per day) colonization with *B. fragilis* group resulted in a higher BMI. In children with a high-fiber diet, colonization with *B. fragilis* group did not influence BMI. A potential explanation for this loss of association between colonization with the *B. fragilis* group and BMI in the high-fiber consumers might be that consuming a high-fiber diet at the age

Table 5. Generalized estimating equation (GEE) results for colonization and counts (log_{10} CFU g^{-1}) of *Bacteroides fragilis* group at 1 month of age and BMI *z*-score measured repeatedly between 1 and 10 years of age in children with a conventional lifestyle ($n = 618$)

		Crude β (95% CI)	P-*value*	*Adj β (95% CI)*[a]	P-*value*	*Adj β (95% CI)*[b]	P-*value*
Low-fiber diet (< 15.0 g)[c]							
Prevalence of colonization, *n* (%)	242 (84%)	0.43 (0.26 to 0.60)	0.00	0.36 (0.18 to 0.53)	0.00	0.34 (0.17 to 0.52)	0.00
Counts (log_{10} CFU g^{-1}), median (range)[d]	9.36 (5.74 to 10.36)	−0.04 (−0.10 to 0.01)	0.14	−0.04 (−0.10 to 0.01)	0.09	−0.05 (−0.10 to 0.00)	0.05
High-fiber diet (> 15.0 g)[c]							
Prevalence of colonization, *n* (%)	179 (81%)	−0.07 (−0.31 to 0.17)	0.56	−0.10 (−0.28 to 0.10)	0.32	−0.10 (−0.31 to 0.09)	0.24
Counts (log_{10} CFU g^{-1}), median (range)[d]	9.39 (5.77 to 10.27)	0.06 (−0.01 to 0.13)	0.07	0.07 (0.01 to 0.14)	0.02	0.08 (0.01 to 0.14)	0.02

Abbreviations: Adjβ, adjusted β; BMI, body mass index; CFU, colony-forming unit; CI, confidence interval. Stratified for fiber intake (low or high intake). Numbers in the table may not add up to 618 because of missing values in dietary intake variables. [a]Adjusted for age BMI measurements, infant gender, birth weight, place and mode of delivery, maternal education level, maternal BMI before pregnancy, duration of breastfeeding, age at collection of fecal sample, type of infant feeding and total bacteria counts. [b]Additionally adjusted for nutrition intake at 4 years of age (total energy intake (kcal), energy from fat (%) and energy from protein (%). [c]Median fiber intake at the age of 4 years is calculated for the total population, including both groups (alternative and conventional). For the conventional recruitment group and alternative recruitment group, the median fiber intake is 14.31 and 16.47 g, respectively. [d]Includes only children who were colonized with the specific bacteria group or species.

of 4 years might compensate for the effect that early *B. fragilis* group colonization has on BMI.

Our results for higher counts of *B. fragilis* group in children who are colonized are less clear. Higher counts are positively associated with BMI only in children in the conventional subcohort and a high-fiber diet, but inversely associated in children with a low-fiber diet, and in children in the alternative subcohort. A plausible explanation might be a different composition of *B. fragilis* group species between infants in the two different recruitments groups. The pregnant women recruited through alternative channels might introduce a different lifestyle for their children, promoting early colonization with other bacteroides species than in the conventional subgroup. Indeed, species within the *B. fragilis* group might differentially affect weight development as suggested by Bervoets *et al.*[22] who showed more *B. fragilis* in obese and more *B. vulgatus* in control subjects. Identification of bacteroides at the species level in future prospective studies is warranted to address whether a different species distribution actually precedes (over) weight development during childhood.

We are not aware of any previous human studies that investigated the interaction between bacteroides and dietary fiber intake. It is important to note that although we were able to study this interaction, collection of fecal samples (at 1 month) and dietary information (at 4 years) did not take place at the same time, and this is clearly a weakness of this study. Despite the high instability of the microbiota in infancy, it may be speculated that the presence of *B. fragilis* group as pioneer species in the neonatal gut could be indicative for the subsequent persistence of these bacteria or other developmental processes toward an adult-like microbiota. Although dietary information was only collected once, at the age of 4 years, we used a validated FFQ to determine the habitual dietary intake. The FFQ reflects the long-term dietary pattern and gives a much better estimate of habitual dietary intake than instruments such as food diaries and 24-h recall. In addition, previous studies showed that dietary patterns stay relatively stable during childhood.[43,44]

Furthermore, we observed that neonatal colonization with *C. difficile* was associated with a lower BMI at the age of 103 ± 5 months. As asymptomatic carriage of *C. difficile* is very common in the first years of life and a significant effect was only found in the children in the conventional subcohort, after adjustment of potential confounders, and merely in the last time point of BMI measurement (average age of 105 months), caution in drawing conclusions is required. As we conducted several tests for the five different bacteria groups/species, a type I error because of multiple testing cannot be excluded. Therefore, further research is required to replicate our findings.

We did not find an association between bifidobacteria and BMI development. This is in contrast to the results from Kalliomäki *et al.*,[26] who found lower levels of bifidobacteria at 6 and 12 months of age in children with normal weight ($n = 24$) compared with overweight or obesity ($n = 25$) at 7 years of age. That study was the first to show an association between intestinal microbiota composition and development of overweight. In our study, fecal samples were collected only at age 1 month when almost all children are colonized with high concentrations of bifidobacteria, and this might mask a potential effect of bifidobacteria.

The prospective study design, a large study population, adjustment for the main determinants of the microbiota composition in early infancy (for example, place and mode of delivery, type of infant feeding in the first month) and repeated measurements of BMI on 7 occasions over a time period of 10 years are major strengths of our study. Nevertheless, the present study also has some limitations; one drawback is that only five bacterial groups and genera were measured, although others may also be involved in weight development in childhood.[45] Previous studies using extensive profiling of the neonatal gut microbiota, have however shown that the microbiota at this age is still very simple and dominated by the bacterial groups targeted in the present study.[46] Another drawback of this study was the timing of fecal sampling collection at 1 month of age. In early infancy, the gut microbiota composition is relatively unstable and its role in shaping the microbiota at a later age is to date unknown.[47,48] This study only provides information on the role of the intestinal microbiota at early infancy. The measured species might actually indicate presence or absence of other unstudied co-occurring bacterial genera or species that influence weight development.[49] Another limitation relates to the time between collection of the fecal sampling by the parents and analyzing the samples in the laboratory that was 1 day for the majority of samples. Even though the total amount of bacterial DNA, as well as the diversity of the microbiota, may decrease significantly in such a time period, the similarities of the fecal samples processed directly and those processed after 24 h remain high.[36] This also applies for the temperature at which the fecal samples are held in the first 24 h after collection.[50]

Finally, in the present study, we made use of parent-reported body weight and height. Even though the procedure of measuring weight and height of their child was explained and numerous

studies have demonstrated that self-reported questionnaires are a valid method to estimate body weight and height,[51,52] it is known that parents of children with a low BMI tend to overreport body weight, whereas parents of children with a high BMI tend to underreport body weight.[53,54] Recently, we reported a similar underestimation in the KOALA study.[55] This implies that the association we observed between the intestinal microbiota and BMI may actually have been stronger than reported.

CONCLUSION

Our study indicates that presence of *B. fragilis* group in early infancy tended to be associated with a higher BMI later in childhood. If causal, this may have important public health implications as children with an elevated BMI are more likely to remain overweight as adults.[56] Even a moderate increase in BMI over a long period of time has been shown to increase disease risk.[57] Some studies found that a reduction of 0.25 BMI *z*-scores results in a small improvement in metabolic markers among obese adolescents,[58] and a reduction of 0.5 BMI *z*-scores leads to a major improvement.[59] Modification of the composition of the intestinal microbiota could contribute to the prevention of overweight and obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to the children and parents who participated in the KOALA Birth Cohort Study. The following sponsors contributed to data collection for the present study: Netherlands Organisation for Health Research and Development (ZonMw Grant No. 2100.0090), Netherlands Asthma Foundation (Grants No. 3.2.03.48 and No. 3.2.07.022), Netherlands Heart Foundation (Grant No. 2008B112), Triodos Foundation, Phoenix Foundation, Raphaël Foundation, Iona Foundation, Foundation for the Advancement of Heilpedagogiek, Royal Friesland Foods (currently FrieslandCampina); Netherlands Sugar Foundation and the Ministry of Economic affairs, all in The Netherlands. The sponsors had no influence on the analysis and reporting of the present study.

AUTHOR CONTRIBUTIONS

LEJM Scheepers performed the literature search, carried out the statistical analyses, wrote the manuscript with the help of J Penders and ICW Arts and approved the final manuscript as submitted; J Penders contributed to the design of the study and collection of the data, performed the literature search, interpreted the data, contributed to the writing of the manuscript, critically reviewed and revised the manuscript and approved the final manuscript as submitted; C Mbakwa Akwi contributed to the analyses and interpretation of the data, reviewed and revised the manuscript and approved the final manuscript as submitted; C Thijs was the principal investigator and was responsible for the design and conduct of the study, contributed to the collection of the data, critically reviewed the manuscript and approved the final manuscript as submitted; M Mommers contributed to the design of the study and collection of the data, critically reviewed and revised the manuscript and approved the final manuscript as submitted; ICW Arts performed the literature search, interpreted the data, contributed to the writing of the manuscript, critically reviewed and revised the manuscript and approved the final manuscript as submitted.

REFERENCES

1 Bastien M, Poirier P, Lemieux I, Despres JP. Overview of epidemiology and contribution of obesity to cardiovascular disease. *Prog Cardiovasc Dis* 2014; **56**: 369–381.
2 Lakshman R, Elks CE, Ong KK. Childhood obesity. *Circulation* 2012; **126**: 1770–1779.
3 Schonbeck Y, Talma H, van Dommelen P, Bakker B, Buitendijk SE, Hirasing RA *et al.* Increase in prevalence of overweight in Dutch children and adolescents: a comparison of nationwide growth studies in 1980, 1997 and 2009. *PLoS One* 2011; **6***e27608*.
4 Raoult D. Obesity pandemics and the modification of digestive bacterial flora. *Eur J Clin Microbiol Infect Dis* 2008; **27**: 631–634.
5 Farooqi S, O'Rahilly S. Genetics of obesity in humans. *Endocr Rev* 2006; **27**: 710–718.
6 Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I *et al.* Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006; **118**: 511–521.
7 Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; **102**: 11070–11075.
8 Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027–1031.
9 Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022–1023.
10 Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. *PLoS One* 2009; **4**: e7125.
11 Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE *et al.* A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480–484.
12 Zuo HJ, Xie ZM, Zhang WW, Li YR, Wang W, Ding XB *et al.* Gut bacteria alteration in obese people and its relationship with gene polymorphism. *World J Gastroenterol* 2011; **17**: 1076–1081.
13 Nadal I, Santacruz A, Marcos A, Warnberg J, Garagorri M, Moreno LA *et al.* Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. *Int J Obes* 2009; **33**: 758–767.
14 Xu P, Li M, Zhang J, Zhang T. Correlation of intestinal microbiota with overweight and obesity in Kazakh school children. *BMC Microbiol* 2012; **12**: 283.
15 Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C *et al.* Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* 2010; **18**: 190–195.
16 Abdallah Ismail N, Ragab SH, Abd Elbaky A, Shoeib AR, Alhosary Y, Fekry D. Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Arch Med Sci* 2011; **7**: 501–507.
17 Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y *et al.* Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci USA* 2009; **106**: 2365–2370.
18 Balamurugan R, George G, Kabeerdoss J, Hepsiba J, Chandragunasekaran AM, Ramakrishna BS. Quantitative differences in intestinal Faecalibacterium prausnitzii in obese Indian children. *Br J Nutri* 2010; **103**: 335–338.
19 Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P *et al.* Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes* 2008; **32**: 1720–1724.
20 Mai V, McCrary QM, Sinha R, Glei M. Associations between dietary habits and body mass index with gut microbiota composition and fecal water genotoxicity: an observational study in African American and Caucasian American volunteers. *Nutr J* 2009; **8**.
21 Karlsson CL, Onnerfalt J, Xu J, Molin G, Ahrne S, Thorngren-Jerneck K. The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* 2012; **20**: 2257–2261.
22 Bervoets L, Van Hoorenbeeck K, Kortleven I, Van Noten C, Hens N, Vael C *et al.* Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathog* 2013; **5**: 10.
23 Million M, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P *et al.* Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. *Int J Obes* 2012; **36**: 817–825.
24 Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008; **88**: 894–899.
25 Santacruz A, Marcos A, Warnberg J, Marti A, Martin-Matillas M, Campoy C *et al.* Interplay between weight loss and gut microbiota composition in overweight adolescents. *Obesity (Silver Spring)* 2009; **17**: 1906–1915.
26 Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008; **87**: 534–538.
27 Luoto R, Kalliomaki M, Laitinen K, Delzenne NM, Cani PD, Salminen S *et al.* Initial dietary and microbiological environments deviate in normal-weight compared to overweight children at 10 years of age. *J Pediatr Gastroenterol Nutr* 2011; **52**: 90–95.
28 Vael C, Verhulst SL, Nelen V, Goossens H, Desager KN. Intestinal microflora and body mass index during the first three years of life: an observational study. *Gut Pathog* 2011; **3**: 8.

29 White RA, Bjornholt JV, Baird DD, Midtvedt T, Harris JR, Pagano M *et al.* Novel developmental analyses identify longitudinal patterns of early gut microbiota that affect infant growth. *PLoS Comput Biol* 2013; **9**: e1003042.
30 Diamant M, Blaak EE, de Vos WM. Do nutrient-gut-microbiota interactions play a role in human obesity, insulin resistance and type 2 diabetes? *Obes Rev* 2011; **12**: 272–281.
31 Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; **489**: 242–249.
32 Samuel BS, Shaito A, Motoike T, Rey FE, Bäckhed F, Manchester JK *et al.* Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci USA* 2008; **105**: 16767–16772.
33 Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM *et al.* Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA* 2004; **101**: 1045–1050.
34 Kummeling I, Thijs C, Penders J, Snijders BEP, Stelma F, Reimerink J *et al.* Etiology of atopy in infancy: the KOALA Birth Cohort Study. *Pediatr Allergy Immunol* 2005; **16**: 679–684.
35 Bastiaanssen JM, de Bie RA, Bastiaenen CH, Heuts A, Kroese ME, Essed GG *et al.* Etiology and prognosis of pregnancy-related pelvic girdle pain; design of a longitudinal study. *BMC Public Health* 2005; **5**: 1.
36 Ott SJ, Musfeldt M, Ullmann U, Hampe J, Schreiber S. Quantification of intestinal bacterial populations by real-time PCR with a universal primer set and minor groove binder probes: a global approach to the enteric flora. *J Clin Microbiol* 2004; **42**: 2566–2572.
37 Rinttila T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J Appl Microbiol* 2004; **97**: 1166–1177.
38 Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology* 2002; **148**: 257–266.
39 Kwaliteitsinstituut voor Gezondheidszorg CBO. Diagnostiek en behandeling van obesitas bij volwassenen en kinderen, 2008.
40 Dutman AE, Stafleu A, Kruizinga A, Brants HA, Westerterp KR, Kistemaker C *et al.* Validation of an FFQ and options for data processing using the doubly labelled water method in children. *Public Health Nutr* 2011; **14**: 410–417.
41 Stichting Nederlands Voedingsstoffenbestand. Nederlands voedingsstoffenbestad 2001 (Netherlands Food Composition Table 2001) 2001.
42 Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A *et al.* The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**: 15718–15723.
43 Singer MR, Moore LL, Garrahie EJ, Ellison RC. The tracking of nutrient intake in young children: the Framingham Children's Study. *Am J Public Health* 1995; **85**: 1673–1677.
44 Bjelland M, Brantsaeter AL, Haugen M, Meltzer HM, Nystad W, Andersen LF. Changes and tracking of fruit, vegetables and sugar-sweetened beverages intake from 18 months to 7 years in the Norwegian Mother and Child Cohort Study. *BMC Public Health* 2013; **13**: 793.
45 Kallus SJ, Brandt LJ. The intestinal microbiota and obesity. *J Clin Gastroenterol* 2012; **46**: 16–24.
46 Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS *et al.* Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* 2013; **185**: 385–394.
47 Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; **5**: 1556–1573.
48 Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M *et al.* Diversity of the human intestinal microbial flora. *Science* 2005 *308* 1635–1638.
49 Rousseau C, Levenez F, Fouqueray C, Dore J, Collignon A, Lepage P. Clostridium difficile colonization in early infancy is accompanied by changes in intestinal microbiota composition. *J Clin Microbiol* 2011; **49**: 858–865.
50 Cardona S, Eck A, Cassellas M, Gallart M, Alastrue C, Dore J *et al.* Storage conditions of intestinal microbiota matter in metagenomic analysis. *BMC Microbiol* 2012; **12**: 158.
51 Spencer EA, Appleby PN, Davey GK, Key TJ. Validity of self-reported height and weight in 4808 EPIC-Oxford participants. *Public Health Nutr* 2002; **5**: 561–565.
52 Nyholm M, Gullberg B, Merlo J, Lundqvist-Persson C, Rastam L, Lindblad U. The validity of obesity based on self-reported weight and height: implications for population studies. *Obesity (Silver Spring)* 2007; **15**: 197–208.
53 Scholtens S, Brunekreef B, Visscher TL, Smit HA, Kerkhof M, de Jongste JC *et al.* Reported versus measured body weight and height of 4-year-old children and the prevalence of overweight. *Eur J Public Health* 2007; **17**: 369–374.
54 Shields M, Connor Gorber S, Janssen I, Tremblay MS. Obesity estimates for children based on parent-reported versus direct measures. *Health Rep* 2011; **22**: 47–58.
55 Timmermans SH, Mommers M, Gubbels JS, Kremers SP, Stafleu A, Stehouwer CD *et al.* Maternal smoking during pregnancy and childhood overweight and fat distribution: the KOALA Birth Cohort Study. *Pediatr Obes* 2013.
56 Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes Rev* 2008; **9**: 474–488.
57 Baker JL, Olsen LW, Sorensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. *N Engl J Med* 2007; **357**: 2329–2337.
58 Ford AL, Hunt LP, Cooper A, Shield JP. What reduction in BMI SDS is required in obese adolescents to improve body composition and cardiometabolic health? *Arch Dis Child* 2010; **95**: 256–261.
59 Reinehr T, de Sousa G, Toschke AM, Andler W. Long-term follow-up of cardiovascular disease risk factors in children after an obesity intervention. *Am J Clin Nutr* 2006; **84**: 490–496.
60 Santacruz A, Collado MC, García-Valdés L, Segura MT, Martín-Lagos JA, Anjos T *et al.* Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br J Nutr* 2010; **104**: 83–92.