- KOALA -

A longitudinal birth cohort study exploring the impact of alternative lifestyles on childhood development and atopic disorders.

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About the study:

The main focus of the KOALA Birth Cohort Study is to identify key factors that influence the development of atopy in infants and children. Exposures of interest include parental and child lifestyle, including anthroposophy, vaccinations, antibiotics, dietary habits, breastfeeding and breast milk composition, intestinal microbiota composition, infections during the first year post gestation and epigenetics.[1]

The KOALA longitudinal prospective birth cohort study includes 2,500 children born in 2001 and 2003 in the southern Netherlands. The research is conducted by Maastricht University, with principle investigators including Carel Thijs, John Penders, Monique Mommers, Annette Stafleu, Lucy Pond, Stef Kremers, Pieter Dagnelie, Jessica Gubbels, Carolina Moltó Puigmarti and PhD students Ester Sleddens, Catherine A Mbakwa and Marianne Eijkemans.

A total of 2,834 pregnant women were recruited for the study. Recruitment began in 2000 with 2,343 women selected from within an existing prospective cohort study on pelvic girdle pain for the 'conventional lifestyle' group. An additional 491 women were recruited for the 'alternative lifestyle' group through organic food shops, anthroposophic physicians and midwives, Steiner schools and dedicated magazines.[1]

KOALA

A Dutch acronym for *child, parents and health: lifestyle and genetic constitution.*

Women with 'alternative' lifestyles were selected to study how various lifestyle choices such as child rearing practices, organic food consumption, vegetarian diets, and limited or no use of vaccinations and/or antibiotics impacted child development and atopy.[1]

The KOALA study combines both social science and biomedical approaches, including biomarkers for genetic studies.

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Follow-up & data collection

KOALA participants will be followed throughout their lifetime. Key exposures were determined using repeated questionnaires for all members of the cohort during pregnancy and during the first two years of the infant's life at 3, 7, 12, and 24 months post-partum. The questionnaires collected information regarding hygiene, infections, nutritional data, child rearing, and other lifestyle characteristics.[1]

Biological samples were also collected, including maternal blood sampling at 36 weeks gestation for to determine IgE, infant feces at 1 month postpartum to determine gut microbiota composition, breast milk samples at 1 month post-partum to determine fatty acid content and immunological markers, infant capillary blood samples at one year and venous blood at two years for determination of infection serology and measurement of IgE. DNA for genetic analysis was collected through Bucal swab from both parents and infant for all participants.[1]

To determine allergy development in infants, parents completed the International Study of Asthma and Allergies in Childhood questionnaire when infants were 7, 12, 24 months old. Nurse home visits were also conducted at 2 years to observe allergic dermatitis.[1]

For a more complete list of questionnaire data and biological samples collected, please visit:
http://www.birthcohorts.net/bch2/?action=show&UserID=143

Baseline Characteristics[1]

	Alternative	Conventional
Parent history of allergies	73.5%	66.5%
Parents with asthma	25.9%	29.2%
Avg. age of mother	33.7yrs	31.7yrs
Mother with higher education	73.0%	42.7%
Intention to breastfeed	93.4%	70.9%
Conventional vaccinations	53.6%	95.0%
Infant not vaccinated	30.9%	1.8%
Infant antibiotic use	13.8%	24.1%
Pets in home	45.6%	46.0%
Exclusive breastfeeding at 4 months	60.3%	23.0%
Diet incl. organic fruit / baby food	56.3% /54.1%	5.4% /7.8%

Birth outcomes collected: birth weight, birth length, gestational age at birth, apgar score, sex, congenital malformation.

Key child data & biological samples[2]

	Child age (years) at sampling									
	<1	1	2	3	4	5	6	7	8	9
Passive smoking	X	X	X			X	X	X	X	X
Breast feeding	X	X	X							
Diet	X	X	X			X	X	X	X	X
Physical activity	X		X			X	X	X	X	X
Medicine intake	X	X	X			X	X	X	X	X
Vaccinations	X	X	X			X				
Asthma/Allergy	X	X	X			X	X	X	X	X
Weight	X	X	X			X	X	X	X	X
Height	X	X	X			X	X	X	X	X
Whole Blood	X		X						X	

Gut microbiota:

The gut microbiota, the community of commensal and symbiotic microorganisms that live in the human intestines, are a key exposure of interest in this birth cohort study primarily due to the novelty of the research.

The ecology of the gut microbiome has been linked to multiple health outcomes including colorectal cancer, diabetes, obesity, atopy and inflammatory bowel syndrome and much more.[3] Research related to the gut microbiome from the KOALA birth cohort has provided the following key insights for healthy infant gut development:

1. **Birth mode and gut microbiome**[4]
   Birth mode plays a significant role in the development of healthy gut micriobiome. Infants born at term, vaginally and at home have higher counts of beneficial gut microbiota.

2. **Infant diet and gut microbiome**[4]
   Infant diet plays a significant role in the development of healthy gut micriobiome. Infants exclusively breastfed have higher counts of beneficial gut microbiota.

3. **Gut microbiome and allergies**[5]
   There is an association with certain bacteria in the infant gut and the development of eczema, recurrent wheeze and allergic sensitization.

Want more details?

Check out the last page for two examples from the KOALA birth cohort of studies on infant gut microbiota.

Over 56 publications

...with multiple exposures and outcomes related to maternal and child health. Key exposures and outcomes[2] are listed below:

EXPOSURE	OUTCOME
Alternative lifestyle	Maternal weight, smoking during pregnancy, hypertension
Bacterial lysate supplement to infant, birth mode, breastfeeding, birth order	Development of atopic dermatitis, microbiota of gut
Breast feeding, feeding on demand	Body mass index (BMI), overweight, weight gain
Breastfeeding	Eczema
CD14 single nucleotide polymorphisms (SNPs)	Breast milk sCD14, atopic development
Complementary feeding, consumption of cows milk	Eczema, recurrent wheeze, any sensitization, sensitization against cow milk, hen egg, peanut; atopic dermatitis; inhalant allergen (at least one)
Delivery method, breastfeeding, formula feeding, hospitalization, premature birth, antibiotic use, siblings	Bacterial counts for specific bacteria
Diet-related restrictive parenting practices	Dietary Intake of 2yr old children, children's behavioral style, child BMI
Dietary sources of trans fatty acids	Trans fatty acids in breast milk
Eating routines, physical activity	Weight status, BMI, overweight, obesity
Energy-balance related parenting practices	Child's diet, activity behavior, BMI
FADS gene variants; maternal fish/fish-oil intake	Plasma DHA, breast milk DHA
Fatty acid in breast milk	Atopic eczema and allergic sensitization in infancy
Fish intake during pregnancy	Birth weight, gestational age
Folic acid supplementation; intracellular folic acid levels	Childhood atopic disease
Gut microbiota	Atopic disease
Length of breastfeeding, maternal allergies	Atopic disease
Mother's organic dairy and meat consumption	Conjugated linoleic acid (CLA) and trans-vaccenic acid (TVA) in human breast milk
Organic food consumption	Atopic disease
Physical activity, sedentary lifestyle, dietary intake	BMI, asthma, overweight
Pre- and post-natal vitamin D supplementation	Childhood lung function

Two example studies from the KOALA cohort:

Factors influencing composition of intestinal microbiota in early infancy[4]

Question studied: What external factors influence the composition of the gut microbiota in early infancy?

Description of study and results: The study examined fecal samples of 1032 infants at one month old to identify specific bacteria using PCR assays, including *Bifidobacteria, E. coli, C. difficile, Bacteriodies, Lactobacilli* and total bacterial count. Univariate and multivariate analysis was used to determine associations with a variety of factors based on maternal questionnaires.

The study found an association between cesarean section deliveries and lower counts of *Bifidobacteria* and *Bacteroides* and higher counts of *C. difficile* compared with vaginally born infants. Infants fed formula, exclusively, had higher counts of *E. coli*, *C. difficile*, *Bacteroides*, and *Lactobacilli*, compared with breastfed infants. In premature and hospitalized infants, *C. difficile* counts were higher. Infants given antibiotics had lower counts of *Bifidobacteria* and *Bacteroides* and infants who had siblings had higher counts of *Bifidobacteria,* compared with infants without siblings.

Ultimately, the study showed that infants who were born at term, vaginally and at home had the highest count of beneficial bacteria.

Strengths: This was one of the first prospective studies on gut microbiota development with such a large sample size and diversity of potential determinants.

Weaknesses: DNA analysis from fecal samples was delayed by one day, which decreases the amount of bacterial DNA and the diversity of the microbiota, however, the levels of micriobiota of most interest were stable.

The role of intestinal microbiota in the development of atopic disorders[5]

Question studied: What role do intestinal microbiota of infants play in the development of allergies?

Description of study and results: The study examined fecal samples of 957 infants using PCR assay. Blood samples for total and specific IgE were collected at 2 years of age, as well as information on atopic symptoms via maternal questionnaire. At two years of age, clinical diagnosis of atopic manifestation was also made.

The study showed that infants with higher counts of *E. coli* has increased risk of developing eczema, with a dose-response relationship. Infants with higher counts of *C. difficile* were also at higher risk of developing eczema, recurrent wheeze, and allergic sensitization. The presence of *C. difficile* was associated with higher risk of diagnosis of atopic dermatitis.

Most importantly, the study shows that gut microbiota composition precedes the development of atopy, and that *E. coli* and *C. difficile* were associated with this.

Strengths: This was the first, large-scale prospective study on gut microbiota and atopic diseases and adjusted for multiple confounders. It also strengthened the hypothesis that gut microbiota interfere with our immune system.

Weaknesses: DNA analysis from fecal samples was delayed by one day, which decreases the amount of bacterial DNA and the diversity of the microbiota, however, the levels of micriobiota of most interest were stable.

References: (1) Kummeling I, Thijs C, Penders J, Snijders BE, Stelma F, Reimerink J, Koopmans M, Dagnelie PC, Huber M, Jansen MC, de Bie R, van den Brandt PA. Etiology of atopy in infancy: the KOALA Birth Cohort Study. Pediatr Allergy Immunol. 2005 Dec;16(8):679-84. **(2)** KOALA Birth Cohort Study. BirthCohorts.net. Accessed April 1, 2014 from http://www.birthcohorts.net/bch2/?action=show&UserID=143. **(3)** Kim BS, Jeon YS, Chun J. Current status and future promise of the human microbiome. J. Pediatr Gastroenterol Hepatol Nutr. 2013 Jun;16(2):71-9. **(4)** Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE.Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006 Aug;118(2):511-21. **(5)** Penders J, Thijs C, van den Brandt PA, Kummeling I, Snijders B, Stelma F, Adams H, van Ree R, Stobberingh EE. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. Gut. 2007 May;56(5):661-7.