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Low-fat yoghurt intake in pregnancy associated with increased child asthma and allergic rhinitis risk: a prospective cohort study

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Abstract

Dairy products are important sources of micronutrients, fatty acids and probiotics which could modify the risk of child asthma and allergy development. To examine the association of dairy product intake during pregnancy with child asthma and allergic rhinitis at 18 months and 7 years in the Danish National Birth Cohort, data on milk and yoghurt consumption were collected in mid-pregnancy (25th week of gestation) using a validated FFQ (*n* 61 909). At 18 months, we evaluated asthma and wheeze using interview data. We assessed asthma and allergic rhinitis using a questionnaire at the age of 7 years and through registry linkages. Current asthma was defined as self-reported ever asthma diagnosis and wheeze in the past 12 months. All associations were evaluated using multivariate logistic regression. At 18 months whole milk was inversely associated with child asthma (≥5·5 times/week *v.* none: 0·85, 95 % CI 0·75, 0·97); the reverse was true for semi-skimmed milk (≥5·5 times/week *v.* none: 1·08, 95 % CI 1·02, 1·15). For yoghurt, children of women who ate low-fat yoghurt >1 serving/d had 1·21 (95 % CI 1·02, 1·42) greater odds of a medication-related ever asthma diagnosis compared with children of women reporting no intake. They were also more likely to have a registry-based ever diagnosis and report allergic rhinitis. Low-fat yoghurt intake was directly related to increased risk of both child asthma and allergic rhinitis, while whole milk appeared protective for early-life outcomes only. Nutrient components or additives specific to low-fat yoghurt may be mediating the increase in risk.

Key words: **Cohort studies: Maternal nutrition: Yoghurt: Asthma**

The past few decades have seen a rise in asthma and other allergic diseases. In Denmark alone, allergic asthma has increased almost 2-fold over the past 15 years[(1)]. These diseases now contribute considerably to the chronic disease burden that stretches from childhood to adulthood. As most asthma develops during childhood, it has been hypothesised that factors influencing the *in utero* environment, including maternal diet during pregnancy, may affect immune system development and later clinical disease[(2)].

Numerous dietary factors have been examined in relation to allergic disease outcomes. Since the introduction of the lipid hypothesis by Black & Sharpe[(3)], the interest in fatty acids as aetiological agents in allergic disease development has grown. Essential fatty acids have primarily been speculated to influence the immune system shift either towards or away from Th2 pathways, making the child more or less susceptible to allergic disease[(4)]. Although focus has been on the essential fatty acids *n*-3 and *n*-6, new evidence suggests the importance of other fatty acids. Conjugated linoleic acids (CLA) are fatty acids found in dairy products and ruminant meats as a result of biohydrogenation in the cow rumen. Dairy products have been found to have a good correlation with blood CLA levels,

Abbreviations: CLA, conjugated linoleic acid; DNPR, Danish National Patient Registry; RMPS, Register of Medicinal Products Statistics.

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making them an appropriate marker of CLA intake[5]. CLA have been found to have immunomodulating properties and have been hypothesised to attenuate the development of allergic disease[6–10]. Other ruminant fatty acids, including vaccenic acid, have been shown to suppress airway inflammation and allergic dermatitis in animals[11,12]. In human subjects, breast milk levels of vaccenic acid and CLA were inversely related to allergic dermatitis and allergic sensitisation[9]. Only a few studies have examined dairy product, milk and yoghurt intake during pregnancy in relation to various allergic outcomes[13–17]. Majority of these have been null[13–16] with some showing inverse associations[17] or suggestions of inverse associations for early-life allergic outcomes[13–15]. Trials with supplementation of prebiotics and probiotics, found in yoghurt, in infancy have been shown to be successful for the prevention of eczema[18–22], but not for later asthma and allergic disease development.

To better understand the role of dairy product intake during pregnancy on the development of child allergic disease we examined milk and yoghurt intake during pregnancy in relation to early, later and ever asthma in 7-year-old children.

Materials and methods

Population and study design

The Danish National Birth Cohort (DNBC) is a prospective cohort study with the aim of examining associations between pregnancy and early-life exposures and offspring diseases. Enrolment took place between January 1996 and October 2002. Women were approached by their general practitioner at the first antenatal visit if they were living in Denmark, who could speak Danish and were planning to carry to term. Close to 60 % of all eligible women received an invitation from their general practitioner; among these, 60 % chose to enrol[23]. The women were interviewed over the telephone twice during pregnancy and filled out an food frequency questionnaire (FFQ) around the 25th week of gestation. The women participated in two telephone interviews after birth, when the child was 6 and 18 months old. We followed the mother–child pairs through national registries linked to our data by the unique identity (Central Person Registry; CPR) number provided to all Danish citizens. At the age of 7 years an additional questionnaire was sent to the mothers.

Out of the 101 045 eligible pregnancies, we included only the first child enrolled for each woman (*n* 89 333) in order to avoid dependency among correlated observations. We further excluded multiple births (*n* 87 090). Including only women who answered any of the questions on dairy product intake, the final sample size for the analysis was 61 909. Final sample sizes varied between 13 621 and 45 610 based on participant response rate for the outcomes.

Mothers provided written informed consent on behalf of their children. The Regional Scientific Ethics Committee for the municipalities of Copenhagen and Frederiksberg approved all study protocols, and all procedures were in accordance with the Declaration of Helsinki.

Exposure variables

A validated 360-item semi-quantitative FFQ was completed around gestation week 25; it referred to intake during the previous 4 weeks[24,25]. Dairy product consumption was recorded in eight questions in the FFQ; two of them asked about consumption of yoghurt, in servings per day (including percentage of fat, with/without fruit) and six questions asked about milk consumption (whole milk, 1·5 % milk, 0·5 % milk, skimmed milk, churn buttermilk and chocolate milk) in glasses per day. The FFQ asked about yoghurt with/without fruit to better estimate carbohydrates; here, we also used it to account for potential differences in additives used to compensate for texture and flavour. Assuming that the serving sizes were approximately equal to 200 ml, we aggregated the milk and yoghurt variables to obtain the frequency measures of total dairy intake. We also quantified frequency of milk intake by summing all types of milk and excluding yoghurt. For our analyses, we examined individual types of dairy product as well as total dairy product, total milk and total full-fat and low-fat yoghurt intake.

Outcome measurements

We assessed outcomes both at the 18 months and 7-year follow-up. At the age of 18 months, a telephone interview was conducted where the mothers were asked about child asthma doctor diagnosis, wheeze symptoms and the number of wheeze episodes since birth. We defined asthma at 18 months as self-reported doctor asthma diagnosis. Cases of ever wheeze were based on reporting of wheeze symptoms in the past 18 months. Children were classified with recurrent wheeze if they had >3 episodes in the first 18 months of life compared with having ≤3 episodes or no reported wheeze.

We evaluated asthma and allergic rhinitis diagnosis at the 7-year follow-up by numerous sources. Asthma and allergic rhinitis were assessed through the standardised International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire mailed to the mothers when the child was 7 years old[26,27]. Current asthma at the age of 7 years was defined as a positive response to questions on ever doctor-diagnosed asthma and wheezing symptoms in the past 12 months. Ever allergic rhinitis was defined based on reported doctor diagnosis of hay fever.

We used the mandatory Danish National Patient Registry (DNPR) to assess asthma based on hospital admissions. Admission information has been collected since 1977, with emergency room and outpatient contacts added in 1995. The registry has been well validated against asthma diagnosis from hospital records[28]. We extracted data from the DNPR in August 2010 and linked it to our data using the CPR number. Cases of ever asthma were identified using the International Classification of Diseases, tenth revision (ICD-10) codes for asthma (J45, J45.0, J45.1, J45.8, J45.9, J46 and J46.9). Allergic rhinitis diagnosis was excluded due to the low number of hospitalisation (226/47 677 = 0·5 %).

We also had access to the Register of Medicinal Products Statistics (RMPS) that started in 1995 and contains detailed individual-level dispensary information[29]. We used a previous validation study[30] to identify Anatomical Therapeutic

Chemical codes for the classification of ever asthma cases. All combinations of drugs for obstructive airway disease were used, except for β-2-agonists as liquid, inhaled β-2-agonists only once or inhaled steroid only once.

Covariates

Variables that were evaluated for inclusion in the multivariate model included socio-economic status (high-level proficiency, medium-level proficiency, skilled, unskilled, student, unemployment according to maternal and paternal occupation), maternal age at birth of child (≤20 years, 21–39 years and ≥40 years), parity (nulli- and multiparous), maternal pre-pregnancy BMI (in kg/m^2) (≤18·5, 18·6–24·9, 25·0–29·9, 30·0–34·9 and ≥35·0), maternal smoking during pregnancy (non-smoker, occasional smoker and current smoker), maternal exercise during pregnancy (yes/no), gestational weight gain (g/week), breast-feeding duration (none, 0–1, 2–3, 4–6, 7–9 and ≥10 months), birth weight (in grams), gestational age (in days since the last menstrual period), child sex, maternal and paternal history of asthma and allergies, and total energy intake. We also examined dietary variables as potential indicators of a healthy lifestyle (fruits, vegetables, alcohol, vitamins A, D and E, and Se and Zn intake from diet and supplements).

Statistical analysis

We examined the distribution of covariates across categories of dairy product intake to identify potential confounders. Distributions were age-standardised due to significant differences in dairy product intake across maternal age categories. We used chi-square and partial *F*-tests with a $P < 0{\cdot}10$ as well as *a priori* considerations based on the current literature to determine the final set of model covariates. Covariates suspected to be intermediates on the causal pathways, such as birth weight, gestational age and gestational weight gain, were excluded to avoid overadjusting the model. The final logistic regression model was adjusted for maternal age, smoking, parity, pre-pregnancy BMI, physical activity, breast-feeding, socio-economic status, child sex, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies and energy (in quintiles). Dairy intake was entered as an indicator variable and individual exposure categories compared with the lowest, reference category. Categories were collapsed twice at the higher end to account for scarce data on high consumers. We initially used ≥2·5 times/d as the highest category for milk intake and ≤1 servings/d for yoghurt intake, but combined the two highest categories to increase power. We also combined the two lowest categories for total dairy product and milk intake as few women did not consume any dairy products or milk. Effect estimates changed slightly, but directionality remained the same before and after collapsing for the majority of the dairy variables. We report here estimated OR and 95 % CI for the final collapsed categories. Only predictors with the strongest and most consistent results are presented in the main text and tables. Ordinal values for the exposure categories were entered separately into the models as a continuous variable to evaluate *P*-value for trend. All tests were two-sided and statistical significance was considered at $P < 0{\cdot}05$. The analyses were performed using SAS software (release 9.2; SAS Institute).

Results

Study cohort

A total of 61 909 women answered any question on dairy intake. The majority of women were between the age of 21 and 39 years (98 %), of higher socio-economic position (high-level proficiencies: 23 %) and nulliparous (53 %) (Table 1). Close to 68 % of all women reported a pre-pregnancy BMI within 18·6–24·9 kg/m^2 and the mean gestational weight gain rate was 467 (SD 216) g/week. Approximately a quarter of participants reported any smoking during pregnancy, with 13 % being current smokers during pregnancy. Breast-feeding rates were high, with 60 % of women breast-feeding longer than 7 months. Prevalence of maternal history of asthma and allergies was 9 and 32 %, respectively. Paternal asthma and allergies were reported for 8 and 24 % of participants, respectively. There was a preference for low-fat (≤1·5 %) varieties of dairy products in the cohort. Compared with 5 % for whole milk, 30 % of participants reported drinking semi-skimmed milk ≥1 time/d. Similarly, 7 % of women reported eating >0·5 servings/d of full-fat yoghurt *v.* 15 % for low-fat yoghurt.

We compared participants *v.* non-participants for child asthma at 18 months and 7 years. Compared with the 16 263 participants not included in our analysis for child asthma at 18 months, the 45 610 participants eligible for the present study were of lower socio-economic status (skilled, unskilled, student and unemployed: 46 *v.* 40 %), less likely to be nulliparous (52 *v.* 56 %) and less likely to smoke less during pregnancy (12 *v.* 14 %). Comparing the 48 252 participants without data on current asthma at 7 years, the 13 621 participants with child asthma data were more likely to report maternal asthma (12 *v.* 8 %) and allergy (35 *v.* 31 %). There were also more male children among the participants compared with the non-participants (57 *v.* 50 %).

Predictors of dairy intake

We examined total dairy product intake across age-standardised study participant characteristics (Table 1). Compared with low-frequency (>0 glasses/d) consumers, high-frequency (>5 glasses/d) dairy product consumers tended to be younger and of medium- and skilled-level proficiency. They were also more likely to have a normal to overweight pre-pregnancy BMI, higher gestational weight gain, lower parity, exercise and not smoke during pregnancy and breast-feed for ≥4 months. They reported an overall healthier diet with higher intakes of fruit, vegetables, Se, Zn and lower alcohol intake. They had lower intakes of vitamins A, D and E. Birth weight was generally higher for children whose mothers consumed more dairy products. No patterns were observed for gestational age. Patterns were similar for total milk and total low-fat yoghurt consumers, except that there were more occasional

Table 1. Age-adjusted covariate distribution across categories of maternal dairy product intake during pregnancy in the Danish National Birth Cohort (*n* 61 909)*
(Number of participants, percentages, and means and standard deviations)

Frequency of dairy intake (glasses/d)...			>0		>1–2		>2–3		>3–4		>4–5		>5	
	n	%	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
n			9128		9764		15 512		11 243		6809		9453	
Maternal age† (%)														
21–39 years	60 699	98	97		98		98		98		98		98	
Socio-economic position (%)														
High-level proficiencies	12 727	23	23		25		23		25		21		19	
Parity (%)														
Nullipara	31 206	53	49		52		51		55		55		56	
Pre-pregnancy BMI (%)														
≤18·5 kg/m^2	2552	5	5		5		4		4		4		4	
18·6–24·9 kg/m^2	39 110	68	65		69		68		71		68		67	
25·0–29·9 kg/m^2	11 122	19	19		19		20		18		20		21	
≥30·0	4614	8	10		8		8		7		8		8	
Physical activity during pregnancy (%)														
Yes	22 908	39	35		39		38		42		40		39	
Smoking in pregnancy (%)														
Current smoker	7731	13	15		12		13		9		12		14	
Breastfeeding duration (%)														
≥7 months	26 267	60	56		62		61		64		59		57	
Maternal asthma %)														
Yes	5420	9	10		9		9		9		9		10	
Maternal allergies (%)														
Yes	18 840	32	35		32		31		32		31		31	
Paternal asthma (%)														
Yes	4820	8	9		8		8		8		8		9	
Paternal allergies (%)														
Yes	14 036	24	24		25		24		25		24		23	
Child sex (%)														
Male	31 470	51	50		51		51		52		52		52	
Gestational weight gain (g/week)	42 607		448	228	463	209	461	212	473	205	479	219	481	225
Birth weight (g)	58 762		3533	571	3571	572	3584	560	3591	568	3597	578	3598	597
Gestational age (d)	61 897		280	14	280	14	280	14	280	14	280	15	280	14
Energy intake (kJ/d)	61 579		8816	2550	9295	2419	9689	2377	10321	2433	10725	2540	11813	2899
Fruit intake (g/d)	61 905		311	287	318	248	301	235	337	249	315	258	364	353
Vegetable intake (g/d)	61 905		125	102	131	96	123	90	133	93	127	102	139	131
Alcohol (g/d)	61 579		1·8	2·8	1·8	2·4	1·6	2·2	1·6	2·2	1·4	2·1	1·2	2·0
Total vitamin A (RE/d)	61 579		1777	35 740	1843	38 303	1950	46 280	1292	577	1270	592	1327	9877
Total vitamin D (μg/d)	61 579		9·4	6·7	9·0	5·8	8·8	5·3	9·0	5·1	8·6	5·0	8·9	5·1
Total vitamin E (α-TE/d)	61 579		18·6	27·2	17·2	21·5	16·1	17·5	15·8	14·8	15·1	15·0	14·5	14·1
Total Se (μg/d)	61 579		66·5	29·8	69·0	28·0	70·6	26·9	72·3	26·4	72·3	28·3	74·3	27·1
Total Zn (mg/d)	61 579		18·3	8·2	19·0	7·6	19·7	7·5	20·2	7·5	20·3	7·2	20·9	7·5

RE, retinol equivalents; THE, tocopherol equivalents.
* Values are standardised to the age distribution of the study population.
† Value not age-adjusted.

smokers among the former. The latter were more likely to be of higher level proficiency, have a lower pre-pregnancy BMI and smoke during pregnancy (data not shown). High consumers of full-fat yoghurt tended to be of higher-level proficiency, with lower pre-pregnancy BMI and breast-feed for a longer period of time (data not shown).

Multivariate analysis

Child asthma, wheeze and recurrent wheeze at 18 months follow-up

The prevalence of child asthma, wheeze symptoms and recurrent wheeze at 18 months were 17 % (*n* 7803/45 633), 27 % (*n* 12 259/45 842) and 9 % (*n* 3948/45 745), respectively. After adjustment for potential confounders we found that maternal intake of whole milk was inverse (≥5·5 times/week *v.* none: 0·85, 95 % CI 0·75, 0·97) for doctor-diagnosed asthma (Table 2). Maternal semi-skimmed milk intake was directly associated with asthma (≥5·5 times/week *v.* none: 1·08, 95 % CI 1·02, 1·15) (Table 3). Similar patterns were observed for wheeze symptoms and recurrent wheeze (data not shown). We found no associations for total dairy product or total milk intake (Tables 4 and 5). Full- and low-fat yoghurt intake was not related to either outcome, though we found a suggestive inverse association for full-fat yoghurt and child asthma that reached significance only in the lower intake categories (>0·5–1 *v.* 0 servings/d: 0·88, 95 % CI 0·77, 1·00) (Table 6). A dose–response was present for semi-skimmed milk intake, while any intake of full-fat yoghurt appeared to be protective of child asthma (Tables 3 and 6).

Table 2. Associations between whole milk intake during pregnancy and child asthma in the Danish National Birth Cohort (Odds ratios and 95 % confidence intervals)

		Asthma (18 months) (*n* 44 691)				Current asthma† (7 years – ISAAC) (*n* 13 324)				Ever asthma (DNPR) (*n* 38 285)				Ever asthma (RMPS) (*n* 38,267)			
Whole milk intake		Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**
No consumption	Crude	6522/38 020			0·24	1300/11 249			0·70	1887/32 488			0·003	10 382/32 474			0·21
	Adjusted‡		1·00	Reference	0·03		1·00	Reference	0·63		1·00	Reference	0·08		1·00	Reference	0·33
1 time/month	Crude	163/1008	0·93	0·79, 1·10		39/330	1·03	0·73, 1·44		61/884	1·20	0·92, 1·57		262/885	0·90	0·77, 1·04	
	Adjusted		1·04	0·85, 1·27			0·94	0·60, 1·47			1·45	1·05, 1·99			0·92	0·76, 1·11	
2·5 times/month	Crude	234/1491	0·90	0.78, 1.04		42/411	0·87	0·63, 1·21		75/1282	1·01	0·79, 1·28		364/1283	0·84	0·75, 0·95	
	Adjusted		0·93	0.79, 1.10			0·90	0·59, 1·38			0·97	0·70, 1·33			0·81	0·68, 0·95	
1·5–3·5 times/week	Crude	245/1446	0·99	0·86, 1·13		43/461	0·79	0·57, 1·08		79/1265	1·08	0·86, 1·36		368/1263	0·88	0·77, 0·99	
	Adjusted		1·00	0·85, 1·17			0·84	0·57, 1·25			1·01	0·75, 1·37			0·99	0·84, 1·15	
≥5·5 times/week	Crude	454/2726	0·96	0·87, 1·07		115/873	1·16	0·95, 1·43		175/2366	1·30	1·10, 1·52		768/2362	1·03	0·94, 1·12	
	Adjusted		0·85	0·75, 0·97			1·18	0·90, 1·56			1·24	1·00, 1·54			0·99	0·87, 1·11	

ISAAC, International Study of Asthma and Allergies in Childhood; DNPR, Danish National Patient Registry; RMPS, Register of Medicinal Product Statistics.
* *P* value for trend using ordinal values for the exposure categories.
† Defined as self-reported asthma diagnosis plus wheeze in the past 12 months.
‡ Adjusted for maternal age, smoking, parity, pre-pregnancy BMI, physical activity, breastfeeding, socio-economic status, child sex, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, and energy (in quintiles).

Table 3. Associations between semi-skimmed milk intake during pregnancy and child asthma in the Danish National Birth Cohort (Odds ratios and 95 % confidence intervals)

		Asthma (18 months) (*n* 44 588)				Current asthma† (7 years – ISAAC) (*n* 13 285)				Ever asthma (DNPR) (*n* 38 175)				Ever asthma (RMPS) (*n* 38 158)			
Semi-skimmed milk intake		Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**
No consumption	Crude	3216/18 741			0·01	678/5471			0·03	960/15 835			0·41	4948/15 829			0·02
	Adjusted‡		1·00	Reference	0·02		1·00	Reference	0·13		1·00	Reference	0·69		1·00	Reference	0·19
1 time/month	Crude	145/870	1·00	0·83, 1·20		26/279	0·73	0·48, 1·10		47/777	1·00	0·74, 1·35		246/777	1·02	0·87, 1·19	
	Adjusted		1·03	0·83, 1·28			0·48	0·26, 0·88			1·11	0·76, 1·61			1·10	0·90, 1·34	
2·5 times/month	Crude	426/2651	0·96	0·86, 1·07		77/754	0·80	0·63, 1·03		131/2261	0·95	0·79, 1·15		680/2256	0·95	0·86, 1·04	
	Adjusted		1·03	0·90, 1·17			0·74	0·54, 1·02			1·01	0·80, 1·27			0·95	0·84, 1·08	
1·5–3·5 times/week	Crude	656/3977	0·99	0·90, 1·08		142/1237	0·92	0·76, 1·11		204/3503	0·96	0·82, 1·12		1093/3495	1·00	0·93, 1·08	
	Adjusted		1·02	0·91, 1·14			0·93	0·73, 1·19			1·01	0·83, 1·23			1·03	0·93, 1·14	
≥5·5 times/week	Crude	3269/18 349	1·08	1·03, 1·14		609/5544	0·87	0·78, 0·98		925/15 799	0·96	0·88, 1·06		5141/15 801	1·06	1·01, 1·11	
	Adjusted		1·08	1·02, 1·15			0·88	0·76, 1·02			0·98	0·87, 1·10			1·04	0·98, 1·11	

ISAAC, International Study of Asthma and Allergies in Childhood; DNPR, Danish National Patient Registry; RMPS, Register of Medicinal Product Statistics.
* *P* value for trend using ordinal values for the exposure categories.
† Defined as self-reported asthma diagnosis plus wheeze in the past 12 months.
‡ Adjusted for maternal age, smoking, parity, pre-pregnancy BMI, physical activity, breastfeeding, socio-economic status, child sex, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, and energy (in quintiles).

Table 4. Associations between total dairy product intake during pregnancy and child asthma in the Danish National Birth Cohort (Odds ratios and 95 % confidence intervals)

Total dairy product intake (glasses/d)		Asthma (18 months) (*n* 45 633)				Current asthma† (7 years – ISAAC) (*n* 13 635)				Ever asthma (DNPR) (*n* 39 058)				Ever asthma (RMPS) (*n* 39 040)			
		Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**
>0	Crude	1188/6629			0·76	280/2084			0·02	385/5651			0·24	1888/5646			0·30
	Adjusted‡		1.0	Reference	0·90		1·00	Reference	0·12		1·00	Reference	0·56		1·00	Reference	0·46
>1–2	Crude	1212/7122	0·94	0·86, 1·03		241/2115	0·83	0·69, 1·00		341/6188	0·80	0·69, 0·93		1902/6183	0·88	0·81, 0·96	
	Adjusted		1·02	0·92, 1·13			0·79	0·62, 0·99			0·88	0·72, 1·06			0·95	0·86, 1·05	
>2–3	Crude	1969/11 593	0·94	0·87, 1·01		394/3356	0·86	0·73, 1·01		583/9814	0·86	0·76, 0·99		3064/9817	0·90	0·84, 0·97	
	Adjusted		1·02	0·93, 1·12			0·92	0·75, 1·13			0·99	0·84, 1·18			0·94	0·86, 1·03	
>3–4	Crude	1342/8331	0·88	0·81, 0·96		254/2471	0·74	0·62, 0·88		395/7189	0·80	0·69, 0·92		2199/7183	0·88	0·82, 0·95	
	Adjusted		0·96	0·86, 1·06			0·73	0·58, 0·92			0·88	0·73, 1·06			0·93	0·85, 1·03	
>4–5	Crude	865/5090	0·94	0·85, 1·03		170/1519	0·81	0·66, 1·00		259/4357	0·86	0·74, 1·02		1381/4357	0·92	0·85, 1·01	
	Adjusted		1·01	0·90, 1·14			0·85	0·65, 1·10			0·90	0·73, 1·11			0·94	0·84, 1·05	
>5	Crude	1227/6868	1·00	0·91, 1·09		235/2090	0·82	0·68, 0·98		353/5859	0·88	0·76, 1·02		1985/5854	1·02	0·95, 1·10	
	Adjusted		1·01	0·91, 1·13			0·81	0·63, 1·04			0·94	0·77, 1·15			1·05	0·95, 1·17	

ISAAC, International Study of Asthma and Allergies in Childhood; DNPR, Danish National Patient Registry; RMPS, Register of Medicinal Product Statistics.
* P value for trend using ordinal values for the exposure categories.
† Defined as self-reported asthma diagnosis plus wheeze in the past 12 months.
‡ Adjusted for maternal age, smoking, parity, pre-pregnancy BMI, physical activity, breastfeeding, socio-economic status, child sex, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, and energy (in quintiles).

Table 5. Associations between total milk intake during pregnancy and child asthma in the Danish National Birth Cohort (Odds ratios and 95 % confidence intervals)

Total milk intake (glasses/d)		Asthma (18 months) (*n* 45 520)				Current asthma† (7 years – ISAAC) (*n* 13 600)				Ever asthma (DNPR) (*n* 38 971)				Ever asthma (RMPS) (*n* 38 953)			
		Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**
>0	Crude	1688/9749			0·28	377/2994			0·01	546/8388			0·46	2698/8379			0·04
	Adjusted‡		1·00	Reference	0·67		1·00	Reference	0·10		1·00	Reference	0·68		1·00	Reference	0·26
>1–2	Crude	940/5560	0·97	0·89, 1·06		213/1689	1·00	0·84, 1·20		274/4842	0·86	0·74, 1·00		1496/4840	0·94	0·87, 1·02	
	Adjusted		1·04	0·94, 1·16			0·98	0·78, 1·23			0·90	0·75, 1·09			0·97	0·88, 1·07	
>2–3	Crude	2610/15 582	0·96	0·90, 1·03		501/4524	0·86	0·75, 1·09		758/13 237	0·87	0·78, 0·98		4144/13 237	0·96	0·91, 1·02	
	Adjusted		1·01	0·93, 1·09			0·90	0·75, 1·08			0·95	0·82, 1·10			0·98	0·91, 1·05	
>3–4	Crude	663/3936	0·97	0·88, 1·07		129/1202	0·84	0·68, 1·03		196/3405	0·88	0·74, 1·04		1028/3402	0·91	0·84, 0·99	
	Adjusted		1·03	0·92, 1·16			0·84	0·63, 1·10			0·87	0·70, 1·09			0·96	0·86, 1·07	
>4–5	Crude	1019/6023	0·97	0·89, 1·06		203/1760	0·91	0·76, 1·09		284/5184	0·82	0·71, 0·96		1663/5183	1·00	0·92, 1·07	
	Adjusted		0·99	0·89, 1·09			0·89	0·70, 1·14			0·82	0·68, 1·00			0·96	0·87, 1·06	
>5	Crude	860/4670	1·08	0·98, 1·18		148/1431	0·80	0·66, 0·98		258/3915	1·01	0·87, 1·18		1360/3912	1·12	1·04, 1·22	
	Adjusted		1·06	0·95, 1·19			0·83	0·63, 1·08			1·09	0·89, 1·33			1·13	1·02, 1·26	

ISAAC, International Study of Asthma and Allergies in Childhood; DNPR, Danish National Patient Registry; RMPS, Register of Medicinal Product Statistics.
* *P* value for trend using ordinal values for the exposure categories.
† Defined as self-reported asthma diagnosis plus wheeze in the past 12 months.
‡ Adjusted for maternal age, smoking, parity, pre-pregnancy BMI, physical activity, breastfeeding, socio-economic status, child sex, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, and energy (in quintiles).

Table 6. Associations between total full-fat yoghurt intake during pregnancy and child asthma in the Danish National Birth Cohort
(Odds ratios and 95 % confidence intervals)

Total full-fat yoghurt intake (servings/d)		Asthma (18 months) (*n* 45 593)				Current asthma† (7 years – ISAAC) (*n* 13 620)				Ever asthma (DNPR) (*n* 39 020)				Ever asthma (RMPS) (*n* 39 002)			
		Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**
0	Crude	6031/34 257			<0·0001	1221/10 355			0·19	1755/29 104			0·10	9543/29 086			<0·0001
	Adjusted‡		1·00	Reference	0·002		1·00	Reference	0·20		1·00	Reference	0·48		1·00	Reference	0·01
>0–0·5	Crude	1311/8278	0·88	0·83, 0·94		251/2359	0·89	0·77, 1·03		415/7190	0·96	0·86, 1·07		2073/7195	0·83	0·78, 0·88	
	Adjusted		0·90	0·84, 0·98			0·85	0·71, 1·03			0·96	0·83, 1·10			0·87	0·81, 0·93	
>0·5–1	Crude	400/2714	0·81	0·73, 0·90		90/801	0·95	0·75, 1·19		124/2404	0·85	0·70, 1·02		702/2399	0·85	0·77, 0·93	
	Adjusted		0·88	0·77, 1·00			0·92	0·68, 1·23			0·89	0·70, 1·13			0·98	0·87, 1·10	
>1	Crude	53/344	0·85	0·64, 1·14		11/105	0·88	0·47, 1·64		19/322	0·98	0·61, 1·56		89/322	0·78	0·61, 1·00	
	Adjusted		0·81	0·56, 1·16			0·99	0·46, 2·11			1·21	0·69, 2·10			0·80	0·58, 1·10	

ISAAC, International Study of Asthma and Allergies in Childhood; DNPR, Danish National Patient Registry; RMPS, Register of Medicinal Product Statistics.
* *P* value for trend using ordinal values for the exposure categories.
† Defined as self-reported asthma diagnosis plus wheeze in the past 12 months.
‡ Adjusted for maternal age, smoking, parity, pre-pregnancy BMI, physical activity, breastfeeding, socio-economic status, child sex, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, and energy (in quintiles).

Further adjustment for other foods (fruit and vegetables) and nutrient (vitamin E, vitamin D, Se, Zn from diet and supplements) intake did not change the results.

Ever asthma and current child asthma at the 7-year follow-up

The prevalence of ever asthma by the DNPR was 6 % (*n* 2316/39 058) and 32 % (*n* 12 419/39 040) by the RMPS. About 12 % (1574/13 635) of children were classified with current asthma. Compared with children whose mothers reported no whole milk consumption, children whose mothers drank whole milk ≥5·5 times/week were 1·24 (95 % CI 1·00, 1·54) more likely to have an ever asthma diagnosis in the DNPR (Table 2). Total milk (>5 glasses/d *v.* none: 1·13, 95 % CI 1·02, 1·26) intake was directly related to an RMPS ever asthma diagnosis (Table 5). No associations were found for semi-skimmed milk, total dairy product and full-fat yoghurt intake. Total low-fat yoghurt was furthermore associated with ever asthma by the RMPS (>1 servings/d *v.* none: 1·21, 95 % CI 1·02, 1·45) (Table 7). The association was also borderline significant for ever asthma by DNPR diagnosis and current asthma at the age of 7 years. A dose–response was suggestive for current asthma at the age of 7 years and ever asthma by the DNPR only. Further adjustment for other foods and nutrient intake did not change the results.

Ever child allergic rhinitis at the 7-year follow-up

Of the children, 5 % (*n* 1887/38 762) were reported to have had an ever hay fever doctor diagnosis. Mothers who consumed >1 serving/d of the total low-fat yoghurt *v.* none were 1·40 (95 % CI 1·00, 1·97) times more likely, respectively, to have children with a self-reported ever allergic rhinitis (data not shown). Further adjustment for other foods and nutrient intake did not change the results.

Sensitivity analyses

Since our initial hypothesis involved ruminant *trans*-fatty acids, we examined their role in these analyses. There were positive correlations between ruminant *trans*-fatty acids and both whole (Spearman *r* 0·23, $P < 0·0001$) and semi-skimmed milk (*r* 0·30, $P < 0·0001$). The fatty acids were weakly associated with full-fat yoghurt (without fruit: *r* 0·07, $P < 0·0001$; with fruit: *r* 0·13, $P < 0·001$) and low-fat yoghurt (without fruit: *r* −0·12, $P < 0·0001$; with fruit: *r* −0·12, $P < 0·001$). Total dairy product intake was not associated with ruminant *trans*-fatty acids (*r* −0·002, $P = 0·71$) and weakly associated with total milk intake (*r* 0·01, $P = 0·01$). Correlations for total full- and low-fat yoghurt were similar to the coefficient for yoghurt with fruit. Ruminant *trans*-fatty acids by themselves were not associated with either the early or later childhood outcomes. Putting the individual dairy product in the same model as the fatty acids did not substantially alter the effect estimates, except for semi-skimmed milk and RMPS ever asthma diagnosis (≥5·5 times/week *v.* none: 1·07, 95 % CI 1·00, 1·14).

Due to the transient nature of asthma during the first few years of life and to better capture clinical asthma, we excluded

Table 7. Associations between total low-fat yoghurt intake during pregnancy and child asthma in the Danish National Birth Cohort
(Odds ratios and 95 % confidence intervals)

Total low-fat yoghurt intake (servings/d)		Asthma (18 months) (*n* 44 260)				Current asthma† (7 years – ISAAC) (*n* 13 182)				Ever asthma (DNPR) (*n* 37 871)				Ever asthma (RMPS) (*n* 37 853)			
		Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**	Cases/*n*	OR	95 % CI	*P**
0	Crude	3164/18 512			0·31	583/5395			0·02	912/15 814			0·14	4996/15 811			0·54
	Adjusted‡		1·00	Reference	0·64		1·00	Reference	0·06		1·00	Reference	0·03		1·00	Reference	0·15
>0–0·5	Crude	3330/19 239	1·02	0·96, 1·07		680/5759	1·11	0·98, 1·24		962/16 380	1·02	0·93, 1·12		5262/16 370	1·03	0·98, 1·08	
	Adjusted		1·05	0·99, 1·12			1·14	0·98, 1·32			1·03	0·91, 1·16			1·07	1·01, 1·14	
>0·5–1	Crude	867/5437	0·92	0·85, 1·00		202/1641	1·16	0·98, 1·37		303/4707	1·12	0·98, 1·29		1440/4703	0·96	0·89, 1·03	
	Adjusted		0·98	0·89, 1·08			1·12	0·89, 1·39			1·20	1·01, 1·42			0·98	0·90, 1·08	
>1	Crude	186/1072	1·02	0·87, 1·20		51/387	1·25	0·92, 1·70		60/970	1·08	0·82, 1·41		341/969	1·18	1·03, 1·35	
	Adjusted		1·06	0·87, 1·28			1·39	0·94, 2·04			1·26	0·90, 1·77			1·21	1·02, 1·45	

ISAAC, International Study of Asthma and Allergies in Childhood; DNPR, Danish National Patient Registry; RMPS, Register of Medicinal Product Statistics.
* *P* value for trend using ordinal values for the exposure categories.
† Defined as self-reported asthma diagnosis plus wheeze in the past 12 months.
‡ Adjusted for maternal age, smoking, parity, pre-pregnancy BMI, physical activity, breastfeeding, socio-economic status, child sex, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, and energy (in quintiles).

DNPR and RMPS diagnoses made prior to the age of 3 years. The number of DNPR cases decreased by about 60 % (*n* 908/37 650) and RMPS cases by 80 % (*n* 2872/29 493). The effect estimates fluctuated and the confidence intervals widened for most relationships. The OR for whole milk with DNPR ever asthma diagnosis was, however, strengthened (≥5·5 times/week *v.* none: 1·54, 95 % CI 1·12, 2·12).

As part of the DNPR, patient-type information is registered (inpatient, outpatient and emergency room). We used this data to evaluate differences in risk of child ever asthma, stratified by patient type. The endpoints for the emergency room were too few to draw meaningful conclusions. The effect estimate for whole milk among children diagnosed by inpatient visit was strengthened (≥5·5 times/week *v.* none: 1·35, 95 % CI 1·02, 1·78). Differential associations were found for total low-fat yoghurt: inpatient (>1 servings/d *v.* none: 1·11, 95 % CI 0·69, 1·78) and outpatient visit type (>1 servings/d *v.* none: 1·54, 95 % CI 0·97, 2·43).

The inconsistency in results among the different types of dairy products prompted us to examine whether these differences may be accounted for by lifestyle factors such as socio-demographic, anthropometric and dietary variables. We therefore looked at changes in these variables with higher intake of any specific dairy product, effectively comparing low-fat *v.* high-fat dairy consumers and milk *v.* yoghurt consumers. We found no consistent patterns except for smoking where high milk consumers tended to smoke during pregnancy while high yoghurt consumers were non-smokers (data not shown).

Discussion

In this large, longitudinal study we used detailed dietary data to examine the relationship between maternal dairy intake during pregnancy and the development of asthma and allergic rhinitis in childhood. We found that results differed by outcomes examined in early *v.* late childhood. Maternal whole milk and full-fat yoghurt intake displayed suggestive protective associations for child outcomes at 18 months; on the other hand, semi-skimmed milk was directly associated with wheeze at 18 months. At the 7-year assessment maternal low-fat yoghurt consumption was directly associated with child asthma and allergic rhinitis; a direct relationship was suggestive for milk intake.

These results do not support the role of ruminant *trans*-fatty acids in allergic disease development, as inverse associations were found across products with different fat content. The inconsistencies we observed may be due to several factors. Timing of the outcome could be important, as associations differed for early and later outcomes. Asthma is a heterogeneous outcome and symptom manifestations may involve different pathologies operating at different time points in childhood. Although wheeze may go on to develop into clinical asthma, early diagnosis is not always reliable and could involve other underlying conditions such as viral infections and bronchitis[31]. These conditions may also never develop into allergic disease but cease as the child grows and lung size increases. The bacterial and viral infections responsible for early wheezing have been shown to be characterised by both Th1 and Th2 responses depending on the specific organism, disease severity and genetic

background[32–34]. Consequently, exposure to different nutrient components in dairy products in fetal life may have an impact on early immune processes differently compared with processes involved in allergic disease development in later childhood, and may involve distinct cellular and molecular components specific to a Th1 or a Th2 mechanism, or both.

The observed relationships may also be dependent on the type of dairy product. At 18 months, the associations differed across percentage of fat for milk but not yoghurt intake. In later childhood, we found that low-fat, but not full-fat, yoghurt was directly related to the outcomes assessed. Furthermore, odds of child asthma increased monotonically across intake categories, with intake exceeding 1 serving/d being of most concern. Dairy products are complex foods that contain a spectrum of nutrients, including SFA and ruminant *trans*-fatty acids, water-soluble vitamins and minerals, such as Mg, Zn and Se. Yoghurt furthermore contains probiotics, and low-fat varieties additives such as artificial sweeteners[35,36]. Studies that have examined dairy product and milk intake during pregnancy in relation to outcomes such as allergic sensitisation[13,14,16,37], wheeze[15,37], asthma[15,16] and lung function[16] have found no association. Only one study in a Japanese birth cohort found a protective relationship between maternal dairy product and milk intake and child wheeze at 16–24 months[17]. Inverse associations have only been found for ruminant *trans*-fatty acids in breast milk with eczema and allergic sensitisation in children <4 years old[9,38]. These largely null observations are consistent with our study results for total dairy product and milk intake, but not the individual dairy products. Therefore, consumption of specific types of dairy products may be more important than total intake. The diversity and complexity of our results make it difficult to interpret and propose a single agent mechanism, yet the consistent associations with low-fat yoghurt for later childhood outcomes suggest that compounds specific to this food, such as artificial sweeteners, may play a role. In Denmark, artificial sweeteners, primarily aspartame, have been present in the food supply since the 1980s[39]. There is some evidence on the role of these additives in relation to inflammation and allergies; however, these findings have been inconsistent and the conclusions unclear[40–43].

Our study is important for the further understanding of prenatal dairy exposure on the development of child asthma and allergic disease. We prospectively followed a large cohort and collected detailed information on maternal dairy product intake during pregnancy and on the child outcomes. For the 7-year outcome assessment, we used both self-reported data and national registries. Our self-reported outcome assessment may be misclassified, but is better at capturing outcomes such as allergic rhinitis, where children are less likely to be hospitalised and use prescription medication, and thereby be identified by the registries, due to more moderate symptoms and use of over-the-counter drugs. The DNPR, though it has complete follow-up, captures only hospitalised cases and the ICD classification could be limited by miscoding. However, a recent validation study in Danish male conscripts against medical examination found that any misclassification in the DNPR was too small to nullify observed associations[44]. We suspect that difference in of disease by reporting method could be of aetiological relevance. In this study, we found differential results by admission type, with results strengthened for low-fat yoghurt exposure for diagnoses made in outpatient settings. This appeared to indicate that the adverse associations may be limited to more moderate asthma. This would also be supported by the low-fat yoghurt results for RMPS ever asthma diagnosis, which is more likely to capture mild/moderate asthma. However, results differed for full-fat yoghurt where a direct association was suggested for DNPR and an inverse association for RMPS diagnosis. This may be due to a small cell number in the highest intake category and an examination of more populated cells in the lower intake categories indicates more consistent results. Differences for whole milk intake could be explained by its relation to disease severity where the direct association for ever asthma by the DNPR was limited to inpatient visits.

There were some limitations to our study. We evaluated current asthma and ever allergic rhinitis at the age of 7 years by self-report; therefore misclassification is plausible. Nonetheless, our definition of current asthma has shown high agreement among non-cases (> 90 %) when compared with the DNPR[45], thereby avoiding false-positive and biased results. Associations with current asthma could have been missed due to small sample size, especially in the highest intake category. We had limited information on child diet and when examining early child milk intake as either a marker of maternal diet or an intermediary on the causal pathway, we found that correlations between maternal and child milk consumption at 18 months and 7 years were weak to modest. When we adjusted for child milk intake at the age of 7 years, effect estimates were strengthened primarily for total intakes with current asthma, RMPS ever asthma and ever allergic rhinitis diagnosis. The association between whole milk and DNPR ever asthma diagnosis weakened slightly (data not shown).

We examined whether intake of specific dairy products could serve as a marker for other lifestyle habits. However, when examining socio-demographic, anthropometric and dietary variables across whole/low-fat milk and yoghurt intake, we found no consistent patterns either for low *v.* full-fat consumers or milk *v.* yoghurt consumers. This suggests that none of the examined variables may explain the observed results, though it is possible that we failed to include all relevant lifestyle factors.

Finally, we always worry about attrition in longitudinal studies due to potential for selection bias. Although a detailed examination of population characteristics in participants *v.* non-participants of this study revealed some differences, they were not large enough to suggest substantial selection bias and were driven by the large sample size. However, we cannot exclude bias remaining from unmeasured covariates and covariates that were poorly measured.

In this study, we found suggestive protective association for maternal whole milk and full-fat yoghurt for child asthma diagnosis at 18 months. Risk of child asthma diagnosis by national registries and self-reported ever allergic rhinitis increased with higher maternal low-fat yoghurt intake. Replication of these results is warranted as is further examination of potential active agents.

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