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Brick, Tabea

2016-06

Brick , T , Schober , Y , Boecking , C , Pekkanen , J , Genuneit , J , Loss , G , Dalphin , J-C , Riedler , J , Lauener , R , Nockher , W A , Renz , H , Vaarala , O , Braun-Fahrlander , C , von Mutius , E , Ege , M J , Pfefferle , P I & PASTURE Study Grp 2016 , ' omega-3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk ' , Journal of Allergy and Clinical Immunology , vol. 137 , no. 6 , pp. 1699-+ . https://doi.org/10.1016/j.jaci.2015.10.042

http://hdl.handle.net/10138/224054
https://doi.org/10.1016/j.jaci.2015.10.042

publishedVersion

ω-3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk

Tabea Brick, BA,[a]* Yvonne Schober, PhD,[b]* Christian Böcking, PhD,[b] Juha Pekkanen, MD,[d] Jon Genuneit, MD, MSc,[e] Georg Loss, PhD,[a,f] Jean-Charles Dalphin, MD, PhD,[g] Josef Riedler, MD,[h] Roger Lauener, MD,[i] Wolfgang Andreas Nockher, MD, MSc,[b] Harald Renz, MD,[b] Outi Vaarala, MD, PhD,[j] Charlotte Braun-Fahrländer, MD,[k] Erika von Mutius, MD, MSc,[a,l] Markus Johannes Ege, MD, MPH,[a,l]* Petra Ina Pfefferle, PhD, DrPH,[b,c]* and the PASTURE study group‡

Munich, Marburg, and Ulm, Germany, Kuopio, Finland, La Jolla, Calif, Besançon, France, Schwarzach, Austria, St Gallen, Davos, and Basel, Switzerland, and Helsinki, Finland

Background: Living on a farm has repeatedly been shown to protect children from asthma and allergies. A major factor involved in this effect is consumption of unprocessed cow's milk obtained directly from a farm. However, this phenomenon has never been shown in a longitudinal design, and the responsible milk components are still unknown.

Objectives: We sought to assess the asthma-protective effect of unprocessed cow's milk consumption in a birth cohort and to determine whether the differences in the fatty acid (FA) composition of unprocessed farm milk and industrially processed milk contributed to this effect.

Methods: The Protection Against Allergy—Study in Rural Environments (PASTURE) study followed 1133 children living in rural areas in 5 European countries from birth to age 6 years. In 934 children milk consumption was assessed by using yearly questionnaires, and samples of the "usually" consumed milk and serum samples of the children were collected at age 4 years. Doctor-diagnosed asthma was parent reported at age 6 years. In a nested case-control study of 35 asthmatic and 49 nonasthmatic children, 42 FAs were quantified in milk samples.

Results: The risk of asthma at 6 years of age was reduced by previous consumption of unprocessed farm milk compared with shop milk (adjusted odds ratio for consumption at 4 years, 0.26; 95% CI, 0.10-0.67). Part of the effect was explained by the higher fat content of farm milk, particularly the higher levels of ω-3 polyunsaturated FAs (adjusted odds ratio, 0.29; 95% CI, 0.11-0.81).

From [a]Dr von Hauner Children's Hospital, Ludwig-Maximilians-Universität Munich; [b]the Institute for Laboratory Medicine, Pathobiochemistry and Molecular Diagnostics, Philipps University of Marburg; [c]Comprehensive Biomaterial Bank Marburg, CBBMR, Medical Faculty, Philipps University of Marburg; [d]the Department of Environmental Health, National Institute for Health and Welfare, and Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio; [e]the Institute of Epidemiology and Medical Biometry, Ulm University; [f]the Department of Pediatrics, University of California, San Diego, La Jolla; [g]the Department of Respiratory Disease, UMR/CNRS 6249 chrono-environment, University Hospital of Besançon; [h]Children's Hospital Schwarzach; [i]Christine Kühne Center for Allergy Research and Education, Davos, and Children's Hospital of Eastern Switzerland, St Gallen; [j]the Institute of Clinical Medicine, University of Helsinki; [k]the Swiss Tropical and Public Health Institute, Basel, and the University of Basel; and [l]Comprehensive Pneumology Center Munich (CPC-M), German Center for Lung Research, Munich.

*These authors contributed equally to the work.

‡The Protection Against Allergy—Study in Rural Environments (PASTURE) study group: Marjut Roponen, Anne Karvonen, Anne Hyvarinen, Pekka Tittanen, Sami Remes (Finland); Marie-Laure Dalphin, Vincent Kaulek (France); Sabina Illi, Martin Depner Bianca Schaub, Michael Kabesch (Germany); Remo Frei, Caroline Roduit (Switzerland), Gert Doekes (The Netherlands).

P.I.P. and C.B. were supported by the European Commission (research grants QLK4-CT-2001-00250) and the Von Behring-Röntgen-Foundation vBRS (grant no. 56-0035). M.J.E. was supported by the European Commission FOOD-CT-2006-31708, and KBBE-2007-2-2-06 and the European Research Council (grant no. 250268). Y.S. was supported by the German Center for Lung Research (DZL) Disease Area, AA 1.3.

Disclosure of potential conflict of interest: T. Brick and M. J. Ege have received grants from the German Federal Ministry of Research (BMBF), the German Research Foundation (DFG), the European Commission, the European Research Council, and FrieslandCampina. J. Pekkanen has received grants from the European Union, the Academy of Finland, the Juho Vainio Foundation, the Päivikki and Sakari Sohlberg Foundation, and the Finnish Cultural Foundation. J. Genuneit has received a grant from the European Commission (FOOD-CT-2006-31708 and KBBE-2007-2-2-06). J.-C. Dalphin has received a grant from Novartis Pharma; has received personal fees from Novartis Pharma, Chiesi, Intermune, GlaxoSmithKline, AstraZeneca, and Boehringer Ingelheim; and has received nonfinancial support from Novartis, GlaxoSmithKline, AstraZeneca, Intermune, Chiesi, Boehringer Ingelheim, and Stallergenes. R. Lauener has received grants from the Kühne Foundation and the European Union; serves on advisory boards for Nestlé, Novartis, Meda, Menarini, MSD, and Allergopharma; and has received payment for lectures from Vifor. O. Vaarala is an employee of AstraZeneca R&D. E. von Mutius has received grants from the German Federal Ministry of Education and Research (BMBF), the European Commission, the European Research Council, the German Research Foundation (DFG), and Friesland-Campina; is an Associate Editor for the *Journal of Allergy and Clinical Immunology*; is on the Editorial Board of the *New England Journal of Medicine*; and has consultant arrangements with GlaxoSmithKline, Novartis, Astellas, Pharma Europe, and Vifor Pharma.

Members of the PASTURE Study Group: A. Karvonen and P. Tiittanen have received grants from the Academy of Finland (grant 139021), Juho Vainio Foundation, Kuopio University Hospital (VTR), the Farmers' Social Insurance Institution (Mela), and the Finnish Cultural Foundation. M.-L. Dalphin has received payment for lectures from Merck Sharp & Dohme and has received travel support from Stallergenes. B. Schaub has received grants from the German Research Foundation (DFG), the German Federal Ministry of Education and Research (BMBF), the European Union, and LMU. M. Depner and S. Illi have received grants from the European Research Council. M. Kabesch has received grants from the European Union, the German Ministry of Education and Research, and the German Research Foundation and has received payment for lectures from the European Respiratory Society, the European Academy of Allergy and Clinical Immunology, the American Thoracic Society, Novartis, GlaxoSmithKline, and Bencard. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 12, 2015; revised September 18, 2015; accepted for publication October 8, 2015.

Available online January 12, 2016.

Corresponding author: Markus Johannes Ege, MD, MPH, Dr von Hauner Children's Hospital, Ludwig Maximilian University, Munich, Germany, Lindwurmstr 4, D-80337 München, Germany. E-mail: markus.ege@med.lmu.de.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2015.10.042

Conclusion: Continuous farm milk consumption in childhood protects against asthma at school age partially by means of higher intake of ω-3 polyunsaturated FAs, which are precursors of anti-inflammatory mediators. (J Allergy Clin Immunol 2016;137:1699-706.)

Key words: *Allergy protection, farm milk effect, ω-3 fatty acid, asthma*

Currently, there are no effective preventive measures for asthma and allergies, but there is natural prevention. An example can be found in children growing up on farms, who are at a significantly lower risk for asthma, allergic rhinoconjunctivitis, and atopic sensitization than children living in the same rural area but not directly living on farms. This protective "farm effect" has been shown in many populations and is sustained into adult life.[1,2] The farm exposures contributing to the reduced risk of asthma and allergies have been identified as contact with livestock and animal feed and consumption of unprocessed cow's milk.[3-5] The latter effect is of particular interest because it has been found to work similarly in children from nonfarming families[6-8]; this suggests that a general population might equally benefit from the consumption of unprocessed cow's milk or its native ingredients as well.

Commercially available cow's milk is usually heat treated for inactivating potentially hazardous microorganisms. Thereby other thermolabile milk ingredients, such as proteins, are altered chemically, which might in part explain the loss of the beneficial farm milk effect after pasteurization.[9] Dairy processes also affect the milk lipid fraction because centrifugation and homogenization modify content, balance, and bioavailability of milk fatty acids (FAs).[10] This is important because consumption of milk fat–containing products, such as full-cream milk and butter, has been implied in the protective effect on asthma.[6,11] Likewise, we have previously observed in the Protection Against Allergy—Study in Rural Environments (PASTURE) study that maternal consumption during pregnancy of unskimmed cow's milk and homemade butter affected the fetal immune system in that cord blood mononuclear cells of the exposed neonates produced more of the allergy-protective cytokine IFN-γ on stimulation with mitogens.[12]

Previously, only cross-sectional studies examined the effect of milk consumption on asthma. PASTURE offered the opportunity to step beyond the cross-sectional design and analyze the effect from a longitudinal point of view. The aims of the present analysis were (1) to evaluate the protective effect of farm milk consumption on asthma, (2) to disentangle the effects of heat treatment and alteration of fat composition, and (3) to evaluate the possible role of specific components of milk fat. For the latter, we assessed FA composition in milk samples usually consumed by asthmatic children and healthy control subjects in a nested case-control design.

Abbreviations used

aOR: Adjusted odds ratio
BDR: Bronchodilator response
FA: Fatty acid
hsCRP: High-sensitivity C-reactive protein
OR: Odds ratio
PASTURE: Protection Against Allergy—Study in Rural Environments
PUFA: Polyunsaturated fatty acids

METHODS

Study design and population

PASTURE is a prospective birth cohort study conducted in rural areas of 5 European countries: Germany, Austria, Switzerland, Finland, and France.[13] The study was approved by local research ethics committees in each country, and written informed consent was obtained from the children's parents. Women were recruited during the last trimester of pregnancy (Fig 1). Women living on an animal husbandry farm were assigned to the farming group (n = 351), and women living in the same area but not on a farm were assigned to the reference group (n = 400). Because in Finland no milk samples were taken, Finish children and those having not completed the follow-up period of 6 years (n = 199) were excluded for later analysis. For measuring milk FA content, a case-control population (1:1.5) consisting of 84 children was selected from all children participating in the milk sampling (n = 517) at age 4 years. This case-control population contained all asthmatic patients with available milk samples (n = 35) and a random sample of healthy children (n = 49) exceeding the cases by about 50%. Cases were defined as children having a lifetime diagnosis of asthma once or obstructive bronchitis at least twice, as reported by the parents at the age of 6 years (n = 35), and control subjects were defined as children without such a diagnosis (n = 49).

Measurements

The questionnaires were based on items of the International Study of Allergy and Asthma in Childhood, the Allergy and Endotoxin study, the Prevention of Allergy—Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle study, and the American Thoracic Society questionnaire. Socioeconomic and lifestyle factors, agricultural exposures, and respiratory and other health factors of these women, their husbands, and their children were assessed through questionnaires during pregnancy and regularly up to age 6 years. At the age of 4 years, samples of the children's "usually" consumed milk were taken in the nested case-control sample. A short questionnaire accompanying the milk sample collected data on type of milk. Milk types were defined as follows: farm milk (cow's milk directly derived from a traditionally husbanded farm), shop milk (any cow's milk bought from a shop or supermarket), unprocessed farm milk (farm milk consumed exclusively without any prior boiling), and boiled farm milk (farm milk normally boiled before consumption). Furthermore, the milk fat content was dichotomized at 3.5%, which is consistent with the usual definitions of whole milk in Europe and the United States.[14,15] Thus high-fat milk was defined as shop milk with a fat content of at least 3.5% or farm milk without skimming.[13] At the same time, serum samples were taken from children and quantified for high-sensitivity C-reactive protein (hsCRP) values as a marker of inflammation.[16] At age 6 years, FEV_1 and bronchodilator response (BDR; relative change of at least 12% in FEV_1 after versus before administration of a short-acting β-agonist) were measured, as previously described.[17] Combination variables reflecting functional airway obstruction and reversibility were defined as follows: asthma with FEV_1 greater than or less than the median (1.22 L) and asthma responding or not responding to a bronchodilator with 12% improvement in FEV_1.

FA assessment in cow's milk samples

Characteristics of milk samples are given in Table E1 in this article's Online Repository at www.jacionline.org. After collection, the milk samples were stored at −80°C and thawed shortly before measurement. All samples were measured in duplicates according to the method of Bocking et al.[16] FAs were extracted from 100 μL of cow's milk with 2 mL of MeOH containing internal standard (C18iso, 250 mg/L) and 1 mL of chloroform. After 5 minutes of mixing, again, 1 mL of chloroform and 1 mL of NaCl solution (0.9%) were added to facilitate separation of the phases, followed by 10 minutes of centrifugation at 2100*g*. The chloroform phase was transferred to a fresh vial and evaporated with nitrogen at 37°C until dry. Afterward, the extract

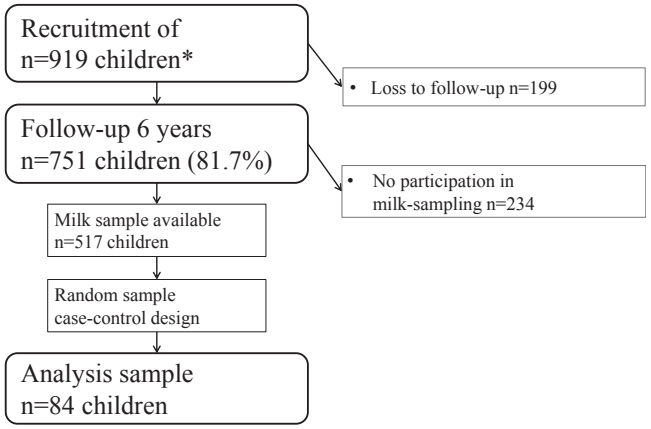

FIG 1. Selection of study population. *Exclusion of Finnish children because the milk module was not implemented in Finland.

was dissolved in 2 mL of hexane. Derivatization of the FAs was performed by adding 0.3 mL of a 2N KOH/MeOH solution and mixing for 5 minutes. The reaction was stopped with the addition of 0.5 g of $NaHSO_4$, followed by another centrifugation step for 5 minutes at 1100*g*. The upper phase containing the FA methyl esters was transferred to a fresh vial and again evaporated under nitrogen. The residue was dissolved in 250 μL of hexane. A panel of 42 FAs was determined by using gas chromatography coupled to a mass spectrometer (for detailed quality controls, see the Methods section in this article's Online Repository at www.jacionline.org). Quantities of FAs were given as arbitrary units approximately corresponding to milligrams per liter. After validation in preliminary analyses, arbitrary unit values were entered in the models without further adjustment for the total amount of FA. FA arbitrary units were summed up within FA groups defined by their chemical properties: saturated FAs, monounsaturated FAs, polyunsaturated fatty acids (PUFAs), ω-3 PUFAs, ω-6 omega-6 PUFAs, trans-FAs, and conjugated linoleic acids.

Statistical analysis

For all statistical analysis, R 3.1.0 software (R Core Team, 2014) was used. To discover disparities in the populations, the children in the recruitment population, the follow-up population, and the analysis sample were compared with respect to socioeconomic and nutritional characteristics by using the Fisher exact test. Milk consumption patterns were compared between 2 subsequent years by using the Pearson correlation coefficient. Logistic regression models were applied to estimate odds ratios (ORs) with 95% CIs for doctor-diagnosed asthma and consumption of different milk types in the follow-up population and analysis sample. Because of the skewed distributions of some FA variables, all FA variables were used after rank transformation for mutual comparisons. The variables for ω-3 and ω-6 PUFAs followed a log-normal distribution and were used log-transformed. The proportion of the respective milk effects on asthma explained by distinct FA groups was quantified by using the change-in-estimate method. Correlation between distinct ω-3 PUFA species and their group variable were assessed by using Spearman rho. *P* values of less than .05 were considered statistically significant. hsCRP values were classified into 3 categories: nondetectable values (<0.20 mg/L), less than the median of detectable values (0.81 mg/L), and greater than the median of detectable values.

RESULTS

The selection process of the population is illustrated in Fig 1. In the follow-up population breast-feeding for at least 6 months was more common and smoking during pregnancy was less common compared with the recruitment population (see Table E2 in this article's Online Repository at www.jacionline.org). As expected,

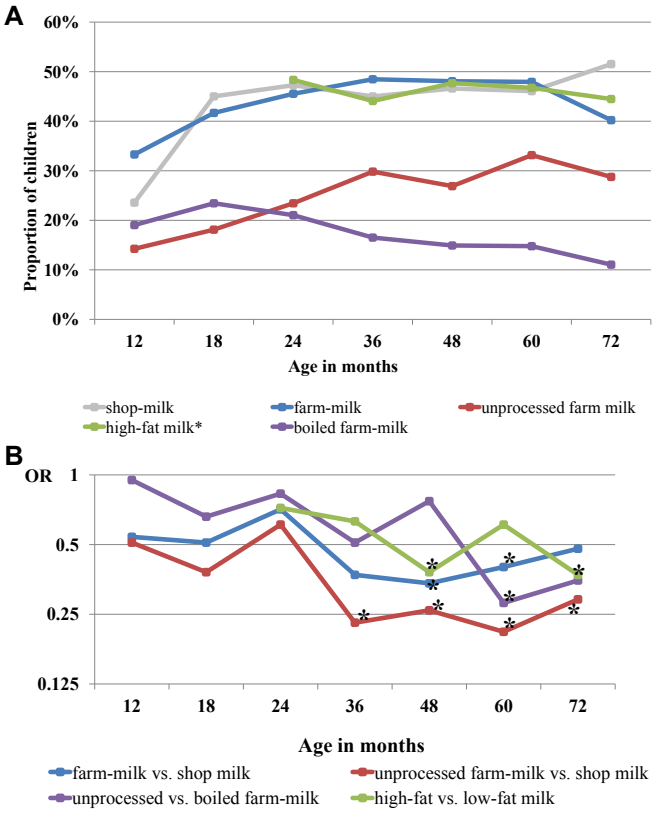

FIG 2. Frequency of milk types consumed over time **(A)** and effects on asthma (in the follow-up population, n = 751; **B**). Fig 2, *A*, Frequency of consumption of milk types from age 1 year until age 6 years. *No data on fat content of consumed milk was collected until age 2 years. Fig 2, *B*, Effects of consuming different milk types at different time points on asthma as defined by age 6 years. ORs were adjusted for center and farming because of the study design. *Significant values, $P < .05$.

children with and without asthma differed with respect to sex, family history of asthma, and milk consumption (see Table E3 in this article's Online Repository at www.jacionline.org). In the analysis sample the proportion of children with a doctor's diagnosis of asthma was increased by design, and consequently, children consuming shop milk were enriched (see Table E2); asthma cases were more exposed to smoking during pregnancy (see Table E3).

In the follow-up population overall consumption of farm milk increased from 1 to 3 years and decreased slightly after 5 years (Fig 2, *A*). At the age of 2 years, consumption of unprocessed farm milk became increasingly more common and replaced successively boiled farm milk; consumption of high-fat milk did not change with age. The high correlations with the preceding years (see Fig E1 in this article's Online Repository at www.jacionline.org) indicate a rather constant consumer behavior with respect to milk type. The effects on asthma of drinking unprocessed farm versus shop milk, unprocessed versus boiled farm milk, or high-fat versus low-fat milk tended to increase with age (Fig 2, *B*); the adjusted odds ratio (aOR) of unprocessed milk versus shop milk consumption decreased, for example, from 0.51 (95% CI, 0.15-1.73) at 1 year to 0.29 (95% CI, 0.11-0.76) at 6 years of age (see Table E4 in this article's Online Repository at www.jacionline.org). The mutually adjusted effects on asthma of unprocessed farm milk versus shop milk and high-fat versus low-fat milk consumption over time were 0.50 (95% CI,

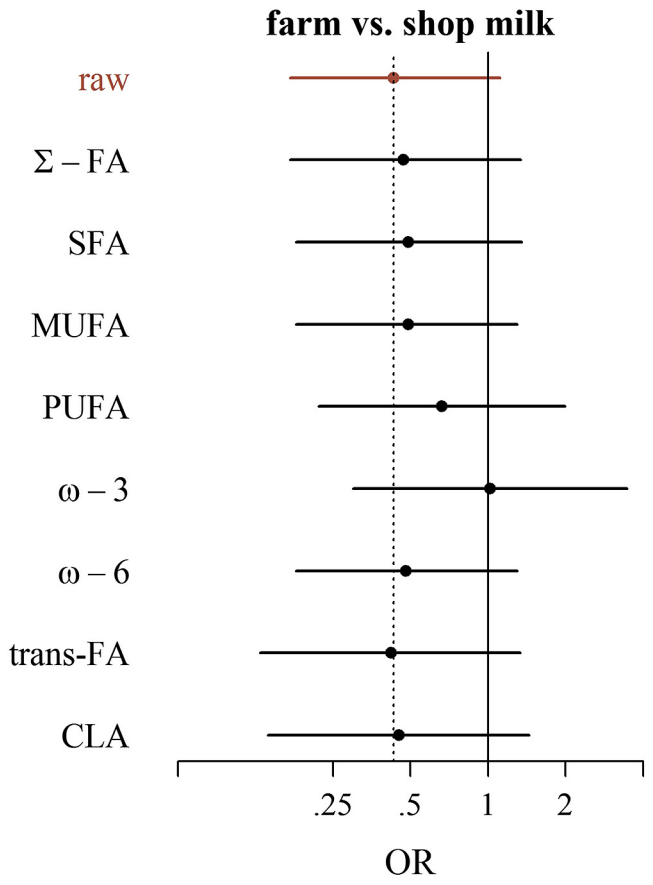

FIG 3. Change in estimate of the farm milk effect on asthma by distinct FA groups. Because of skewed distributions of some FA variables, all FA variables were used after rank transformation. *CLA*, Conjugated linoleic acid; *MUFA*, monounsaturated fatty acid; *SFA*, saturated fatty acid.

0.25-0.98) and 0.60 (95% CI, 0.36-1.01), respectively. As shown in Table E5 in this article's Online Repository at www.jacionline.org, mutual adjustment of both effects led to a reduction of the respective estimates by about 20%, which means that 20% of both effects overlap. The protective effect of high-fat milk tended to be stronger for asthmatic patients with FEV_1 greater than the median or a positive BDR, respectively, although the sample size did not allow for formal confirmation of heterogeneity of effects (see Table E6 in this article's Online Repository at www.jacionline.org). In contrast, the effect of heating was not related to disease severity, as reflected by FEV_1 and BDR (data not shown).

The case-control sample for the analysis of FA contents showed associations similar to those for the follow-up population, thereby showing its representativeness (see Fig E2 in this article's Online Repository at www.jacionline.org). When adjusting the association of farm milk and asthma for the FA groups (Fig 3), the ω-3 group had the strongest change in estimate (111%, see Table E7 in this article's Online Repository at www.jacionline.org). The ω-3 PUFA contents differed significantly between cases and control subjects, with asthmatic children consuming milk with a 35% poorer ω-3 PUFA content (geometric mean ratio, 0.658; $P = .001$; Fig 4). Moreover, the inverse association of ω-3 PUFAs with asthma (aOR, 0.29; 95% CI, 0.11-0.81) was not explained by any potential confounder (see Table E8 in this article's Online Repository at www.jacionline.org).

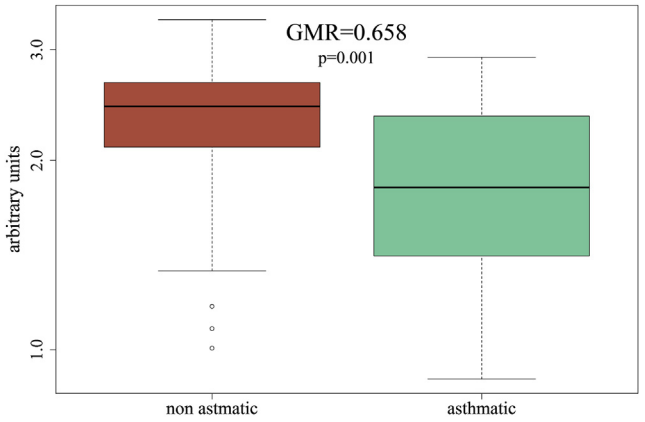

FIG 4. ω-3 PUFA levels (log-transformed) in milk samples consumed by asthmatic and nonasthmatic children and geometric mean ratio *(GMR)*. The GMR was calculated because of log-normal distribution of ω-3 levels.

The overall fat content of unprocessed farm milk was 4.0%, on average, with only a few families (15.6%) skimming the milk before consumption. Conversely, consumption of skimmed or semiskimmed shop milk (<3.5%) was rather common (61.2%). The ω-3 content of unprocessed farm milk was substantially higher compared with that of full-fat shop milk ($P = .03$), which again exceeded the ω-3 content of low-fat shop milk considerably ($P = .0001$; Fig 5, *A*). In contrast, the ω-6 content was only marginally related to the overall fat content (data not shown), resulting in a profoundly skewed ω-6/ω-3 ratio of 3.38:1 in low-fat milk compared with 1.75:1 in high-fat milk (see Table E9 in this article's Online Repository at www.jacionline.org). Yet among high-fat milk samples, the ω-6/ω-3 ratio was affected by thermal treatment and industrial processing, with a significant trend from unprocessed over boiled farm milk to shop milk ($P = .015$; Fig 5, *B*). The ω-6/ω-3 ratio of milk samples collected at age 4 years was positively associated with hsCRP values in serum measured at the same age ($P = .038$, see Fig E3).

DISCUSSION

Regular unprocessed milk consumption was inversely related to asthma onset by age 6 years. The association was stronger with recent exposure compared with exposure in early childhood. This protective effect of native milk was explained partly by absent heating and partly by a higher fat content. The effect of fat content was largely attributable to higher ω-3 PUFA levels and a lower ω-6/ω-3 ratio in unprocessed milk compared with industrially processed milk. The inverse effect of ω-3 PUFA contents on asthma itself was strong and not explained by any potential confounder.

The asthma-protective effect of farm milk consumption in childhood is well in line with findings from several other countries in and beyond Europe.[1,2,5-7,9,18-20] This milk effect is independent from other farm-related exposures, such as animal shed visits, and it is not confounded by family size, family history of atopy, or any other known confounder.[5]

Cow's milk is consumed predominantly by young children, for whom it serves as a breast milk replacement after weaning. Therefore the protective effect of farm milk has previously been attributed to consumption in infancy.[20] Moreover, an anti-infectious effect of unprocessed milk during the first year of life has been found in the PASTURE cohort.[21] However, our present

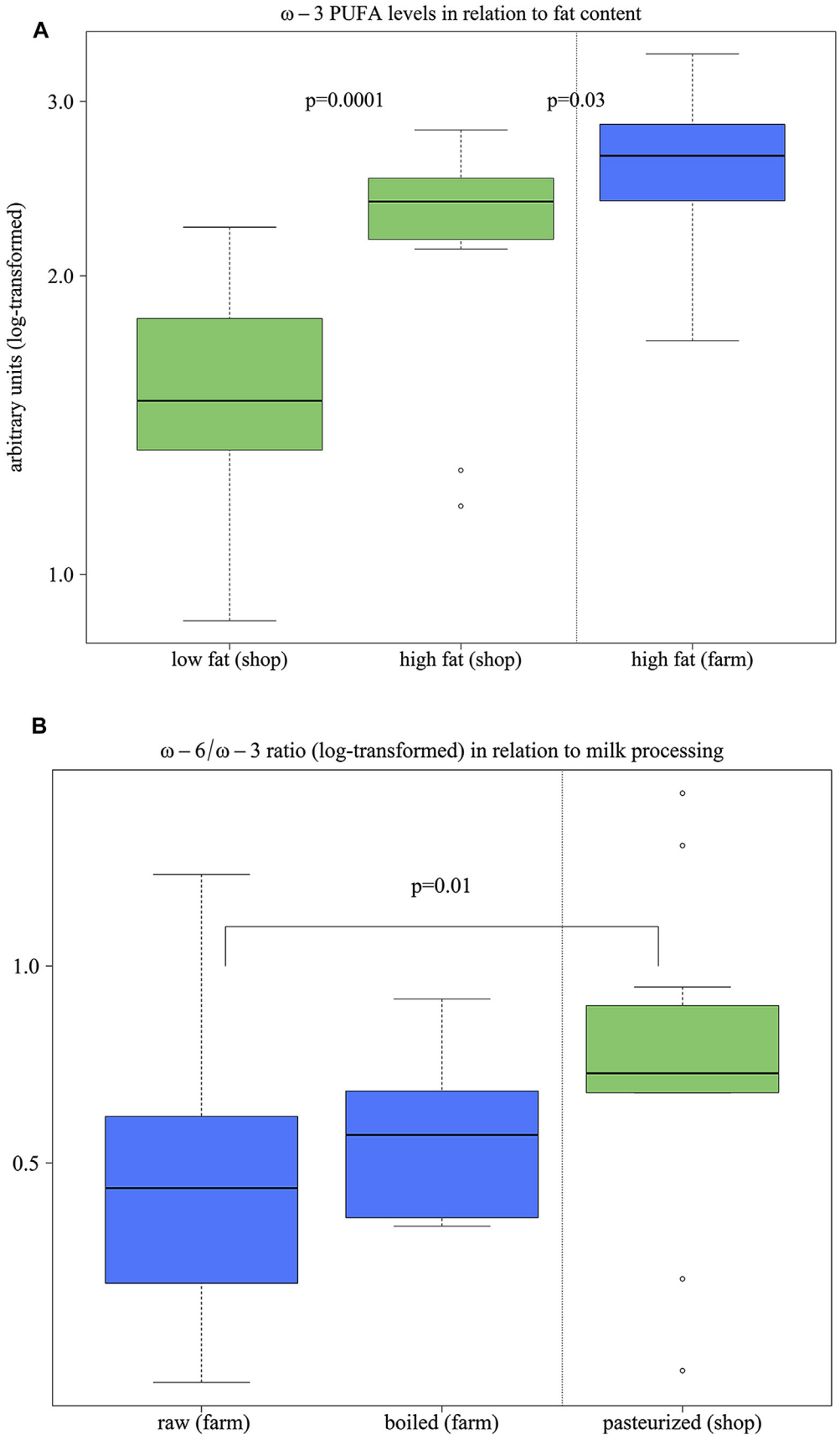

FIG 5. ω-3 PUFA levels in milk samples consumed by PASTURE children **(A)** and ω-6/ω-3 ratio in relation to milk processing **(B)**. Fig 5, *A*, Different ω-3 PUFA levels (log-transformed) in milk samples "usually" consumed by PASTURE children. Fig 5, *B*, Different ω-6/ω-3 ratios (log-transformed) in milk samples "usually" consumed by PASTURE children in relation to milk processing. In Fig 5, *A*, the *t* test (2-sided) was used to calculate differences between milk variables. Fig 5, *B*, shows differences among high-fat milk samples. The *P* value refers to a trend test.

analyses suggest that at least the beneficial effect on asthma increases over time. Recent consumption of farm milk seems to be more relevant than consumption in the first years of life, which extends the concept of early prevention to sustained prevention until school age and beyond. An alternative explanation for the increasing effect size might be found in the growing prevalence of farm milk consumption with age. Nevertheless, the proportion of full-fat milk consumption remained stable over time, although its effect strengthened. Thus this phenomenon invalidates the explanation by growing prevalence.

The discrepancy between the effects of heating and fat content brings us to the following question: What is the critical difference between unprocessed farm milk and industrially processed milk that drives the effect? Essentially, industrial processing involves centrifugation, homogenization, and heat treatment. Obviously, the latter process affects thermolabile milk components, such as microorganisms, whey proteins,[9] or microRNA. Alteration of microRNA content and composition is demonstrated in a separate article.[22] Because farm milk is not homogenized but shop milk generally is, it is difficult to disentangle the effects of homogenization and heat treatment. Centrifugation removes particles, microorganisms, and somatic cells; however, its main goal is to regulate the fat content of the final product. The fat content of native cow's milk varies with breed, feeding, and regional origin[23] and reaches values of 6% or greater, whereas the content of commercially available milk is usually adjusted to 3.5%, 2%, or 1.5%.

Thus a further aim of our analysis was to allocate the farm milk effect to the respective procedures involved in industrial milk processing. The answer to this question was somewhat ambiguous because both fat content and heating exerted strong independent effects on asthma. Consumption of unprocessed versus boiled farm milk clearly mattered, thereby supporting an important role of thermolabile ingredients (Fig 2, *B*). Moreover, adjustment for fat content weakened the effect of unprocessed farm versus shop milk on asthma substantially (see Table E5). This corresponds well to previous findings of inverse associations between asthma and the consumption of butter or full-fat farm milk.[6,11,12]

In contrast to heating, the fat content was associated with disease severity: high-fat milk exerted a somewhat stronger effect on asthma with an FEV_1 of greater than the median or a positive BDR, respectively, compared with asthma with an FEV_1 of less than the median or a negative BDR, respectively (see Table E6). The limited sample size precludes formal evidence of effect heterogeneity; nevertheless, this tendency was consistent over time, thus implying a systematic difference. The more pronounced effect of milk fat on milder forms of asthma suggests a more susceptible phenotype that might be alleviated by a natural form of symptomatic treatment in contrast to more severe asthma phenotypes.[17]

Therefore we were particularly interested in the composition of the fat compartment of cow's milk. Milk fat mainly consists of triglycerides, which comprise esters of the trivalent alcohol glycerol with FAs. The latter vary predominantly with the number of carbon atoms and the proportion of unsaturated bonds between them. Because of limited power, we assessed the FAs in groups of similar chemical properties, such as saturated FAs or PUFAs, or by the distance of the last double bond to the last carbon atom (ie, ω-3 vs ω-6 PUFAs). First, we confirmed that the 35 cases and their control subjects, exceeding them by one and a half times, were only selected for asthma status and related variables, such as family history of asthma (see Table E3). Second, we verified that the associations between milk types and asthma in this analysis population matched those of the entire follow-up cohort (see Fig E2).

TABLE I. Spearman correlation* of single ω-3 PUFAs with the sum of ω-3 PUFAs

	FAs	Correlation
C18.3n3	α-Linolenic acid	0.994
C20.3n3	Eicosatrienoic acid	0.909
C20.5n3	Eicosapentaenoic acid	0.937
C22.4n3	Docosatetratetraenoic acid	0.073
C22.5n3	Docosapentaenoic acid	0.751
C22.6n3	Docosahexaenoic acid	0.624

*Spearman correlation was used because of skewed distributions of some ω-3 PUFA variables.

To figure out which of the FA groups best explained the effect of farm versus shop milk and whether FAs contributed to the effect of fat content and milk processing, we adjusted the respective logistic regression models for the FA groups (Fig 3). In contrast to all other FA groups, only the PUFA group and particularly the ω-3 PUFAs changed the estimate of the effect of farm milk consumption on asthma. We interpret these findings that the ω-3 PUFA group is specifically involved in the protective effect of farm milk consumption on asthma. Hence we assessed the ω-3 PUFA levels in more detail and found a much stronger gradient of ω-3 PUFA levels between high- and low-fat milk compared with ω-6 PUFA levels (see Table E9). This phenomenon might be attributed to 3 factors.

First, the average fat content of the farm milk samples of 4% exceeds the standard value of full cream shop milk by 14%.

Second, most of the farm milk samples in our study were derived from grass-fed or grazing cows, which generally have a lower ω-6/ω-3 PUFA ratio compared with cows fed a mixed diet based on hay, silage, and concentrate.[24] Conversely, shop milk is a blend of various milk batches from all over the European common market with a rather low proportion of milk from pasturing animals.

Third, enzymes metabolizing ω-6 and ω-3 PUFAs differently might be released by mechanical damage to microvesicles or inactivated by industrial processing or heating.[25,26] The latter assumption is suggested by the different ω-6/ω-3 ratios between raw and boiled farm milk (Fig 5, *B*).

The relevant ω-3 PUFA species driving the effect were identified by the highest individual correlations with their group (Table I) as α-linolenic acid (C18.3n3) and its 20-carbon chain derivatives, eicosatrienoic acid (C20.3n3) and eicosapentaenoic acid (C20.5n3). α-Linolenic acid and its ω-6 counterpart, linoleic acid (C18:2n-6), are essential PUFAs metabolized by the same set of enzymes (ie, elongases and Δ5 and Δ6 desaturases; Fig 6). However, ω-3 PUFAs are precursors of anti-inflammatory mediators, whereas ω-6 PUFAs are precursors of proinflammatory mediators. In general, the mentioned enzymes metabolize ω-3 PUFAs with higher affinity than ω-6 PUFAs. In our unprocessed milk samples the overall ω-6/ω-3 ratio of 1.59:1 (see Table E9) was rather favorable with respect to recommended values of 1:1 to 4:1 in foods.[27,28] A lower ω-6/ω-3 ratio limits the metabolization of the proinflammatory prostaglandin D_2 and cysteinyl leukotrienes,[29] although the metabolic pathways might be more complex.[30,31] Ultimately, ω-3 PUFAs interfere with the synthesis of proinflammatory leukotrienes, thereby acting against

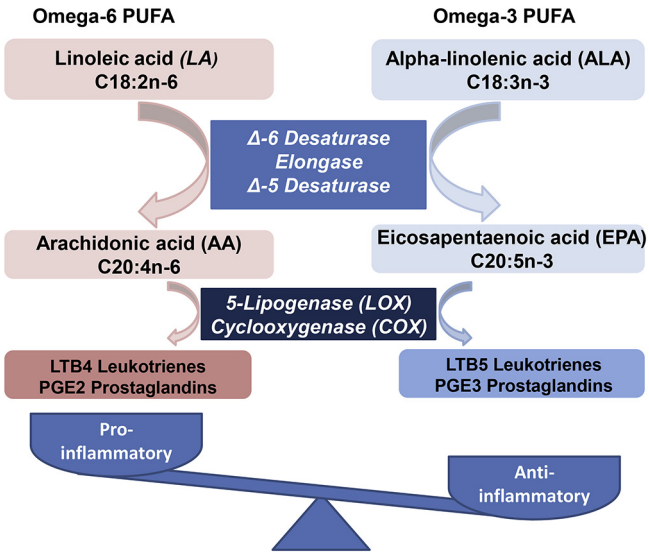

FIG 6. Metabolism of linoleic and α-linolenic acid. In mammalians the PUFA profile is derived from essential FA precursors of both ω-3 and ω-6 PUFAs (α-linolenic acid [18:3 ω-3] and linoleic acid [18:2 ω-6], respectively). Long-chain PUFAs are synthesized endogenously through reactions of both insertion of additional double bonds (desaturases) and elongation of the acyl chain (elongase). ω-3 and ω-6 PUFAs compete for the same set of enzymes in this pathway, with a preferential affinity of ω-3 over ω-6 PUFAs. *LTB*, Leukotriene B; *PGE*, prostaglandin E.

asthma in a similar way as the widely used leukotriene receptor antagonists.[29] Indeed, supplementation with ω-3 PUFAs has been suggested for prevention and amelioration of asthma, allergies, and inflammatory diseases.[27,29,32] Moreover, in our population we found a positive association of the ω-6/ω-3 ratio in milk with levels of serum hsCRP, a marker of low-grade inflammation[16]; this again supports the suggested anti-inflammatory effect of cow's milk.

Admittedly, several trials supplementing mothers during pregnancy or infants during the first years with ω-3 PUFAs failed with respect to prevention of atopic disease.[33-37] However, most of these studies are hampered by a limited duration of the intervention or an insufficient follow-up time. Only one study compared diets enriched for ω-3 versus ω-6 PUFAs starting from 6 months until assessment of asthma at age 5 years. The authors explained the failure of the intervention by insufficient adherence to the protocol. In contrast, in our study the exposure to farm milk was part of the children's usual diet and did not require profound changes in nutritional habits. Nevertheless, we acknowledge that our observational data are not immune to residual confounding, although the specificity of the ω-3 PUFA effect was remarkable.

The major strength of this analysis is the longitudinal study design, with several points of exposure assessment before determining the outcome based on a physician's diagnosis. The restriction of the study population to rural regions of Europe might be considered a potential shortcoming. However, previous studies have shown that the effects of farm milk consumption on asthma and atopy can be found in both suburban and urban settings.[6] In contrast to other farm-related exposures, such as stable visits, the effect of farm milk consumption can be considered as a paradigm for preventive strategies in a general population. Parent-administered questionnaires might be examined with respect to potential bias by social desirability because consumption of unprocessed milk is clearly discouraged. However, parental answers on milk types and mode of milk consumption have previously been validated by objective measures of heat treatment and fat content in a similar population.[9]

In summary, our data demonstrate that continuous consumption of unprocessed farm milk contributes to protection from childhood-onset asthma. To a substantial extent, this effect is attributable to the higher ω-3 PUFA content in unprocessed cow's milk compared with processed shop milk. The advantageous ω-6/ω-3 ratio in cow's milk might shift the metabolic balance of eicosanoid synthesis from proinflammatory to anti-inflammatory mediators, thereby suggesting that the farm milk effect partially consists of an anti-inflammatory treatment of subclinical asthma. Future interventional studies will determine whether fortification of industrially processed milk with ω-3 PUFAs might be a promising approach to primary or secondary prevention of childhood asthma.

We thank Lydia Lerch and Alexandra Fischer for excellent technical assistance. Samples were stored in the Marburg Biobank CBBMR.

Clinical implications: Higher ω-3 PUFA levels, as contained in unprocessed cow's milk, might contribute to natural asthma prevention.

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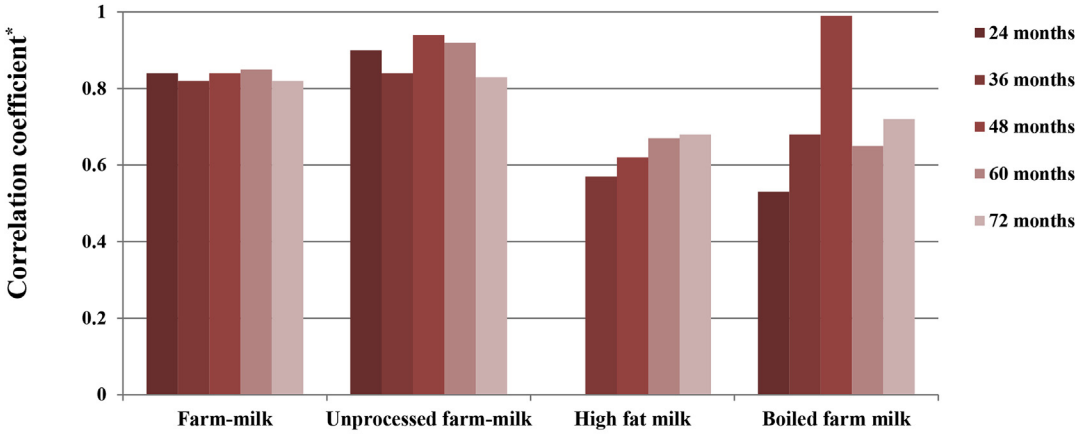
METHODS

Quality control of FA determination

Quality control was conducted as follows. Thawing effects were controlled by establishing comparative measurements in fresh milk samples or samples thawed once or twice, showing that FA pattern and content were not significantly affected by freezing and thawing. For internal quality control, each sample was spiked with C18-iso as an internal artificial standard not occurring in natural sources. For external control, a standard panel containing 42 FAs was measured 2 times per day or at least after the run of 12 samples.



*Correlation coefficient respectively relating to previous year

FIG E1. Correlation of milk consumption over time.

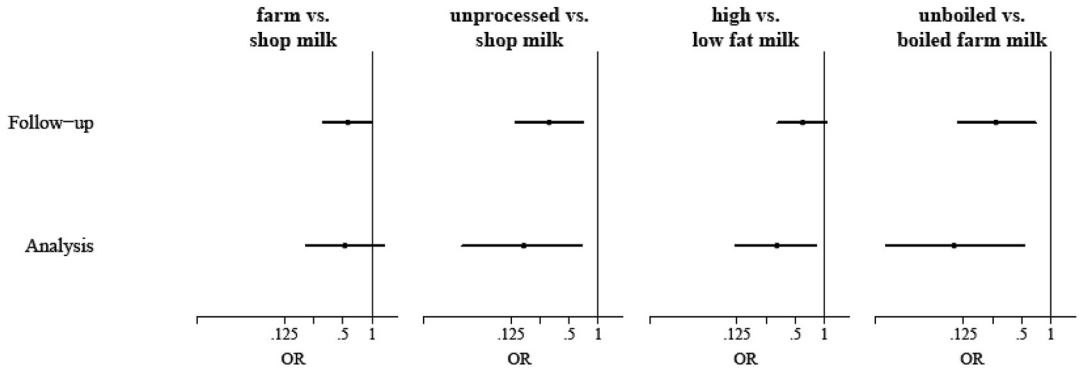
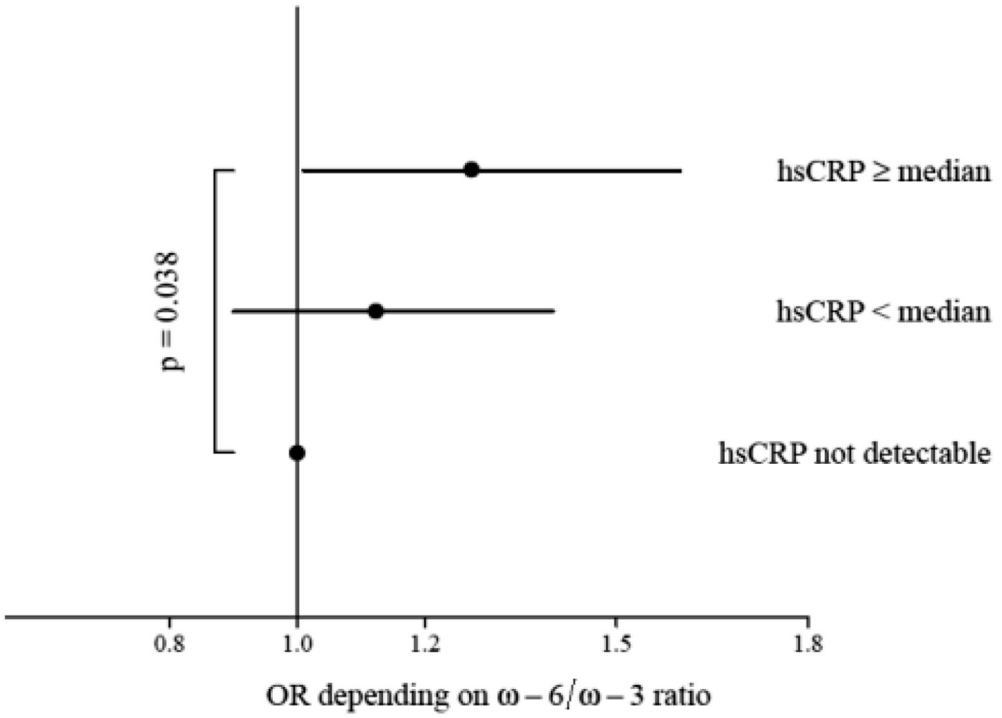


FIG E2. Milk effect in the follow-up population compared with that in the analysis population (5 years).



hsCRP not detectable (detection limit 0.20 mg/L): reference group.

FIG E3. ORs for higher hsCRP values depending on ω-6/ω-3 ratios in consumed milk.

TABLE E1. Characteristics of milk samples assessed in the case-control sample (n = 84)*

	Shop milk (n = 46)			Farm milk (n = 37)	
	Pasteurized (n = 13)	UHT (n = 30)	Other (n = 3)	Raw (n = 25)	Boiled (n = 12)
High fat (≥3.5% fat)	9	4	2	25	12
Low fat (<3.5% fat or skimmed)	2	22	1	0	0
No information about fat content	2	4	0	0	0

*One child provided no information about type of collected milk sample at age 4 years.

TABLE E2. Characteristics of the recruitment, follow-up, and analysis populations

	Recruitment population		Follow-up population			Analysis population		
	n = 919 children	Percent	n = 751 children	Percent	*P* value*	n = 84 children	Percent	*P* value†
Male sex	456	49.6	392	52.3	.63	46	54.8	.65
Farmer	418	45.5	351	46.7	.12	42	50.0	.56
Older siblings	553	60.2	459	62.5	.22	53	63.1	.72
Parental asthma	134	14.6	115	15.3	.79	19	22.6	.05‡
Parental atopy	450	49.0	382	50.9	.71	47	56.0	.66
Breast-feeding ≥6 mo	423	46.0	382	50.9	.01‡	36	42.9	.19
Smoking during pregnancy	125	13.6	86	11.5	.00‡	8	9.5	.72
Cesarean section	213	23.2	155	20.6	.14	16	19.0	.77
Domestic animals (cat/dog)	197	21.4	179	23.8	.66	18	21.4	.59
High parental education§	414	45.0	353	47.0	.36	46	54.8	.16
At age 3 y								
Day care attendance	18	2.0	16	2.1	.69	1	1.2	1.0
At age 5 y								
Milk consumption, never	NA		36	4.8	NA	4	4.8	1.0
Mainly unprocessed farm milk			249	33.2		20	23.8	.06
Mainly boiled farm milk			111	14.8		14	16.7	.18
Mainly shop milk			346	46.1		45	53.6	.06
Mainly milk with a fat content ≥3.5%			351	46.7		37	44.0	.63
Mainly milk with a fat content <3.5%			351	46.7		42	50.0	.63
Stable visits ≥5 times per week	NA		151	20.1	NA	15	17.9	.49
At age 6 y								
Doctor's diagnosis of asthma	NA		57	7.6	NA	35	41.7	.00‡

NA, Not applicable.

**P* values are derived from comparisons of participants (n = 751) and nonparticipants (n = 168) in the follow-up.

†*P* values are derived from comparisons of participants (n = 84) and nonparticipants (n = 667) in the follow-up.

‡Significant difference, $P \leq .05$.

§At least 1 parent with education of greater than 10 years.

TABLE E3. Characteristics between children with and without asthma and cases and control subjects, respectively, in the follow-up and analysis populations

	Study population (n = 751 children)			Analysis population (n = 84 children)		
	Children without asthma (n = 689)	Children with asthma* (n = 57)	*P* value (Fisher exact test)	Control subjects (n = 49)	Cases (n = 35)	*P* value
Male/female sex	350/338	38/19	.03	20/29	26/9	<.01
Farmer/nonfarmer	324/365	25/32	.68	26/23	16/19	.66
Older siblings, yes/no	423/266	34/23	.78	30/19	23/12	.82
Parental asthma, yes/no	94/591	21/33	<.01	5/44	14/19	.01
Parental atopy, yes/no	337/348	39/17	<.01	23/26	24/11	.07
Breast-feeding ≥6 mo/<6 mo	352/312	28/23	.88	21/28	15/15	.64
Smoking during pregnancy, yes/no	976/611	9/46	.27	1/48	7/27	<.01
Cesarean section, yes/no	136/545	10/47	.73	7/41	8/27	.39
Domestic animals (cat/dog), yes/no	168/517	10/45	.33	13/36	5/30	.28
High parental education,† yes/no	320/360	29/26	.48	26/23	20/14	.66
At age 3 y						
Day care attendance, yes/no	14/489	2/35	.30	0/31	1/19	.39
At age 5 y						
Milk consumption, yes/no	648/33	53/3	.75	44/4	35/0	.13
Mainly unprocessed farm milk/shop milk	240/310	8/33	<.01	17/22	3/23	<.01
Mainly farm milk, boiled/unboiled	98/240	12/8	<.01	5/17	9/3	<.01
Mainly milk with a fat content ≥ 3.5%/<3.5%	327/317	20/33	.09	26/18	11/24	.02
Stable per week visits ≥5 times/<5 times	141/173	9/11	1	9/16	6/8	.74

*Five children without information about asthma status.
†At least 1 parent with education of greater than 10 years.

TABLE E4. ORs* and CIs for the effects of consuming different milk types over time (from age 1 to age 6 years) on asthma (age 6 years)

Age of children (mo)	Farm milk vs shop milk, OR (95% CI)	Unprocessed farm milk vs shop milk, OR (95% CI)	Unprocessed vs boiled farm milk, OR (95% CI)	High-fat milk (≥3.5%) vs low-fat milk (<3.5%), OR (95% CI)
12	0.54 (0.20-1.45)	0.51 (0.15-1.73)	0.95 (0.31-2.90)	—
18	0.51 (0.23-1.14)	0.38 (0.13-1.12)	0.66 (0.23-1.89)	—
24	0.71 (0.34-1.51)	0.61 (0.24-1.59)	0.83 (0.33-2.06)	0.72 (0.39-1.33)
36	0.37 (0.18-0.78)	0.23 (0.09-0.59)	0.51 (0.19-1.38)	0.63 (0.33-1.20)
48	0.34 (0.16-0.71)	0.26 (0.10-0.67)	0.77 (0.26-2.32)	0.38 (0.18-0.78)
60	0.40 (0.19-0.85)	0.21 (0.08-0.54)	0.28 (0.11-0.75)	0.61 (0.32-1.15)
72	0.48 (0.22-1.03)	0.29 (0.11-0.76)	0.35 (0.12-1.02)	0.37 (0.19-0.75)

*Adjusted for center and farming.

TABLE E5. Separate and mutually adjusted effects of industrial processing and fat content on asthma

	Separate model		Mutually adjusted model		
	OR (95% CI)	*P* value	aOR (95% CI)	*P* value	CIE*
Unprocessed farm vs shop milk	0.40 (0.20-0.80)	.01	0.50 (0.25-0.98)	.04	24%
High- vs low-fat milk	0.53 (0.34-0.83)	.01	0.60 (0.36-1.01)	.05	20%

CIE, Change in estimate.
*Estimates are averaged over the first 6 years.

TABLE E6. Effect of high-fat milk on asthma (follow-up population) over time

	Effect of high-fat milk on asthma (at age 6 y)			
Age of children (mo)	+ FEV_1 > median (1.22 L), OR (95% CI)	+ FEV_1 < median (1.22 L), OR (95% CI)	+ responding to a bronchodilator by 12% improvement in FEV_1, OR (95% CI)	+ not responding to a bronchodilator by 12% improvement in FEV_1, OR (95% CI)
36	0.21 (0.06-0.74)	0.95 (0.38-2.36)	0.42 (0.13-1.36)	0.57 (0.22-1.44)
48	0.29 (0.09-0.91)	0.53 (0.21-1.31)	0.13 (0.03-0.59)	0.79 (0.31-2.02)
54	0.27 (0.09-0.84)	0.48 (0.18-1.28)	0.25 (0.07-0.86)	0.43 (0.16-1.17)
60	0.35 (0.12-0.97)	1.08 (0.43-2.69)	0.43 (0.13-1.42)	0.67 (0.28-1.59)
72	0.20 (0.06-0.69)	0.58 (0.22-1.53)	0.08 (0.01-0.65)	0.49 (0.19-1.26)

TABLE E7. Farm milk effect on asthma (at age 6 years): raw estimate and after adjustment for FA groups (rank transformed*)

Adjustment	OR (95% CI)	β estimate	CIE	CIE (%)
—	0.43 (0.17 to 1.11)	−0.83	—	—
∑-FA	0.53 (0.19 to 1.49)	−0.63	(−0.83 − [−0.63])/−0.83 = 0.24	24
SFA	0.45 (0.17 to 1.16)	−0.81	(−0.83 − [−0.81])/−0.83 = 0.02	2
MUFA	0.42 (0.15 to 1.13)	−0.87	(−0.83 − [−0.87])/−0.83 = −0.05	−5
PUFA	0.56 (0.21 to 1.52)	−0.58	(−0.83 − [−0.58])/−0.83 = 0.30	30
ω-3 PUFA	1.09 (0.33 to 3.65)	0.09	(−0.83 − [0.09])/−0.83 = 1.11	111
ω-6 PUFA	0.42 (0.16 to 1.10)	−0.87	(−0.83 − [−0.87])/−0.83 = −0.05	−5
Trans-FA	0.40 (0.13 to 1.26)	−0.92	(−0.83 − [−0.92])/−0.83 = −0.11	−11
CLA	0.49 (0.15 to 1.59)	−0.71	(−0.83 − [−0.71])/−0.83 = 0.14	14

CIE, Change in estimate; *CLA*, conjugated linoleic acid; *MUFA*, monounsaturated fatty acid; *SFA*, saturated fatty acid.
*Because of skewed distributions of some FA variables, all FA variables were used after rank transformation.

TABLE E8. Adjustment of the effect of ω-3 PUFA levels on asthma

Exposure	Outcome	Adjustment	OR (95% CI)*
ω-3 PUFA levels (log-transformed)†	Asthma	—	0.29 (0.11-0.81)
		Farm vs shop milk	0.34 (0.10-1.15)
		Unprocessed farm vs shop milk	0.20 (0.04-0.95)
		High-fat vs low-fat milk	0.41 (0.11-1.50)
		Sex	0.30 (0.10-0.89)
		Stable visits at 5 y	0.23 (0.04-1.16)
		Smoking during pregnancy	0.31 (0.10-0.99)
		Breast-feeding ≥6 mo	0.30 (0.10-0.90)

*ORs are shown for separate models assessing 1 confounder at a time.
†Adjusted for center and farming.

TABLE E9. Contents of ω-3 and ω-6 FAs in high- and low-fat milk

	ω-3 PUFAs	ω-6 PUFAs	ω-6/ω-3 ratio
High-fat milk (≥3.5%)	1.18 (1.09-1.28)	2.07 (2.03-2.12)	1.75 (1.66-1.84)
Low-fat milk (<3.5%)	0.66 (0.53-0.79)	2.24 (2.18-2.31)	3.38 (3.25-3.52)
Unprocessed cow's milk	1.34 (1.21-1.47)	2.14 (2.06-2.21)	1.59 (1.47-1.72)
Shop milk	0.76 (0.64-0.89)	2.18 (2.13-2.22)	2.85 (2.72-2.99)

Values represent geometric means of ω-3 and ω-6 FA variables, with 95% CIs in parentheses.