The protective effect of farm milk consumption on childhood asthma and atopy: The GABRIELA study

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Background: Farm milk consumption has been identified as an exposure that might contribute to the protective effect of farm life on childhood asthma and allergies. The mechanism of action and the role of particular constituents of farm milk, however, are not yet clear.

Objective: We sought to investigate the farm milk effect and determine responsible milk constituents.

Methods: In rural regions of Germany, Austria, and Switzerland, a comprehensive questionnaire about farm milk consumption and other farm-related exposures was completed by parents of 8334 school-aged children, and 7606 of them provided serum samples to assess specific IgE levels. In 800 cow's milk samples collected at the participants' homes, viable bacterial counts, whey protein levels, and total fat content were analyzed. Asthma, atopy, and hay fever were associated to reported milk consumption and for the first time to objectively measured milk constituents by using multiple regression analyses.

Results: Reported raw milk consumption was inversely associated to asthma (adjusted odds ratio [aOR], 0.59; 95% CI, 0.46-0.74), atopy (aOR, 0.74; 95% CI, 0.61-0.90), and hay fever (aOR, 0.51; 95% CI, 0.37-0.69) independent of other farm exposures. Boiled farm milk did not show a protective effect. Total viable bacterial counts and total fat content of milk were not significantly related to asthma or atopy. Increased levels of the whey proteins BSA (aOR for highest vs lowest levels and asthma, 0.53; 95% CI, 0.30-0.97), α-lactalbumin (aOR for interquartile range and asthma, 0.71; 95% CI, 0.52-0.97), and β-lactoglobulin (aOR for interquartile range and asthma, 0.62; 95% CI, 0.39-0.97), however, were inversely associated with asthma but not with atopy.

Conclusions: The findings suggest that the protective effect of raw milk consumption on asthma might be associated with the whey protein fraction of milk. (J Allergy Clin Immunol 2011;128:766-73.)

Key words: *Allergic diseases, asthma, atopy, children, farming, hay fever, microorganism, farm milk, risk, whey protein*

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Childhood asthma and allergies remain a major health problem in industrialized countries and increasingly in developing countries.[1] Study populations with a similar genetic background but striking differences in environmental exposures have been especially informative to clarify environmental causes for the onset of asthma and atopy. Studies focusing on differences between rural farming and nonfarming communities have consistently shown that children growing up on a farm are at significantly lower risk of asthma, hay fever, and atopic sensitization than children living in the same rural area but not on a farm.[2]

Environmental factors that have been hypothesized to explain this protective effect of farm life are contact with animals,[3,4] the diversity of microbial exposure,[5] endotoxin levels in house dust,[6] and farm milk consumption.[7-9] Exposure to farm milk in early life[8] and consumption of raw farm milk[7] have been associated with a reduced asthma and atopy risk, and it has been suggested that this protection might be mediated through receptors of the innate immune system.[10]

All previous studies on the effect of farm milk consumption have been questionnaire based and lacked objective measurements of milk components. Hence determination of the biological components associated with a protective farm milk effect is warranted.

The GABRIEL (a multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community) Advanced studies program,[11] comprising a large population of European children, was established to investigate the environmental

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*The members of the GABRIELA study group are shown in Appendix 1.

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Abbreviations used
ALP: Alkaline phosphatase
aOR: Adjusted odds ratio

causes of asthma and atopy and includes data on analytically determined milk constituents. The aim of the present analysis was to find biological components of cow's milk that might explain the protective effect of farm milk on childhood asthma and atopy.

METHODS

Study population and study design

The GABRIEL Advanced studies were conducted in 5 rural areas of southern Germany, Switzerland, Austria, and Poland. Because of differences in study design, the Polish data will be reported separately. In phase I a short recruitment questionnaire was distributed through elementary schools to parents of all 6- to 12-year-old school children in the selected study areas. Three strata were defined as follows: (1) farm children (ie, children living on a farm run by the family); (2) exposed nonfarm children (ie, children not living on a farm but regularly exposed to stables, barns, or cow's milk produced on a farm); and (3) nonexposed nonfarm children. For phase II analyses, a stratified random sample of 9,668 was taken from 34,491 eligible participants. Children whose parents had provided written informed consent for blood sampling, genetic analyses, and dust sampling were eligible (Table I). A comprehensive questionnaire (n = 8,334) provided information about the participants' farm-related exposures, and 7,606 also gave blood samples for IgE measurements.

For more extensive environmental sampling, the study population was restricted to 1 center (Bavaria). Three exclusive disease strata were defined within each exposure stratum: (1) asthma, (2) atopy but no asthma, and (3) no asthma and no atopy. Of the 1903 eligible Bavarian children, 895 were selected by applying disproportionate stratified random sampling to create equally sized samples within each of the 9 strata (the study design is described in more detail elsewhere[11]). Milk samples of 800 subjects were analyzed. The ethics committees of the respective universities and the data protection authorities approved the study.

Atopy

Serum IgE levels against inhalant and food allergens were measured by using a fluorescence immunoassay. Atopy was defined as positive test results for specific IgE antibodies against *Dermatophagoides pteronyssinus*, cat, or birch (cutoff, 0.7 kU/L) or against a grass mix (cutoff, 0.35 kU/L). Food allergy was defined as a positive fx5 test (fish, cow's milk, hen's egg, peanut, soybean, and wheat flour).

Clinical outcomes

Health outcomes were assessed according to International Study of Asthma and Allergies in Childhood standards.[12] Childhood asthma was defined as either wheeze in the past 12 months, asthma inhaler use ever, or a doctor's diagnosis of asthma at least once or wheezy bronchitis more than once. Current asthma was defined as childhood asthma and wheeze in the past 12 months. Hay fever required occurrence of nasal symptoms with itchy or watery eyes in the past 12 months or a doctor's diagnosis of hay fever ever. Atopic dermatitis was defined as a doctor's diagnosis ever.

Milk exposure assessed by means of questionnaire

The phase II comprehensive questionnaire provided information about the child's farm-related exposures. Cow's milk consumption was determined by asking whether the child consumed milk purchased at a shop (shop milk) or directly from a farm (farm milk) and whether farm milk was boiled or skimmed. The heating status of shop milk was not assessed. The parents had to indicate the life period of milk exposure from pregnancy to school age and the corresponding amounts of milk consumption.

Children were grouped into the following categories: (1) exclusive shop milk exposure, (2) mixed milk exposure (exposure to both shop and farm milk), and (3) exclusive farm milk exposure. The information on milk boiling was used to subdivide the farm milk exposure into "only boiled farm milk drinkers" and "any unboiled farm milk drinkers." The latter included children consuming exclusively unboiled farm milk, as well as those consuming both unboiled and boiled farm milk. The "any unboiled farm milk" group was further subdivided by frequency of consumption (daily unboiled farm milk vs less than daily unboiled farm milk) and timing of first unboiled milk exposure (first exposure to unboiled farm milk in the first year of life or during pregnancy vs after 1 year of age).

Milk sample collection and analyses

In phase III trained field workers collected cow's milk that was consumed at the participants' homes on the day of the field visit. Parents were instructed to prepare the milk as they usually did and filled out standardized milk documentation sheets. All samples were analyzed by laboratory staff blinded to the milk type and the health and exposure status.

The heating status of milk samples was defined by the residual activity of the milk indigenous enzymes alkaline phosphatase (ALP) and lactoperoxidase, according to European Commission Council Directive 92/46/EC. Low levels of ALP (<80 mU/L) correspond to milk having been heated to greater than 72°C for at least 15 seconds (minimum for pasteurized milk), and low levels of lactoperoxidase (<20,000 mU/L) correspond to milk having been heated to greater than 85°C for at least 5 seconds (minimum for high heat–treated milk). The measurements and the milk type allowed to categorize the samples as (1) high heat–treated shop milk (≥85°C), (2) pasteurized shop milk (not heated to >85°C), (3) heated farm milk (≥72°C), and (4) raw farm milk (not heated to >72°C). Because 85% of the heat-treated farm milk samples were heated to greater than 85°C, all heated farm milk samples were combined for analysis. The total fat content and whey protein levels were determined for all available phase III samples. For detailed methods, see the Methods section in this article's Online Repository at www.jacionline.org.

Microbiological analyses

The total viable bacterial count was assessed in all 800 milk samples, and 222 samples were selected for advanced microbiological analyses by using stratified random sampling (strata based on milk type, heating status, and fat content). The following microbiological groups were determined by using selective plate count methods: pseudomonades, Enterobacteriaceae, micrococci plus staphylococci, lactobacilli, yeast plus mold, bacilli plus endospores, psychrotropic bacteria, and human pathogens. For detailed methods, see the Methods section in this article's Online Repository.

Statistical analyses

All statistical analyses were performed with STATA/SE 10.1 software for Windows (StataCorp, College Station, Tex). The stratification of the study sample was taken into account by using fixed weights (weighted up to the 34,491 participants eligible for phase II) and the linearized Taylor series method for variance estimation. First, associations between milk exposure and health outcomes were determined in phase II participants by using weighted multivariate logistic regression models adjusting for age, sex, farming status (farmers vs nonfarmers), number of siblings, familial history of asthma or hay fever, study center, and breast-feeding. In sensitivity analyses all final models were adjusted for food allergens (fx5), asthma models were adjusted for atopy, and atopy and hay fever models were adjusted for asthma. An additional adjustment for contact with farm animals or contact with stables and barns was performed to avoid confounding by concomitant farm exposures.

The phase III data were used to explore associations between the objectively assessed heating status of milk or measured milk components and asthma and atopy. These regression models were adjusted for the same set of confounders as the phase II data. Milk type and heating status were categorized into 4 groups, with highly heated shop milk as the reference category. To take into account the distribution of milk constituents with high proportions of nondetectable values (total viable bacterial count, lactoferrin,