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Clinical Commentary Review

The Beneficial Effect of Farm Milk Consumption on Asthma, Allergies, and Infections: From Meta-Analysis of Evidence to Clinical Trial

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The low prevalence of asthma and allergies in farm children has partially been ascribed to the consumption of raw cow's milk. A literature search identified 12 publications on 8 pertinent studies. A meta-analysis corroborated the protective effect of raw milk consumption early in life (<1 to 5 years, according to study) on asthma (odds ratio [OR], 0.58; 95% CI, 0.49-0.69), current wheeze (OR, 0.66; 95% CI, 0.55-0.78), hay fever or allergic rhinitis (OR, 0.68; 95% CI, 0.57-0.82), and atopic sensitization (OR, 0.76; 95% CI, 0.62-0.95). The effect particularly on asthma was observed not only in children raised on farms (OR, 0.62; 95% CI, 0.58-0.82) but also in children living in rural areas but not on a farm (OR, 0.60; 95% CI, 0.48-0.74). This demonstrates that the effect of farm milk consumption is independent of other farm exposures and that children not living on a farm can theoretically profit from this effect. Because of the minimal but real risk of life-threatening infections, however, consumption of raw milk and products thereof is strongly discouraged. Raw farm milk and industrially processed milk differ in many instances including removal of cellular components, manipulation of the fat fraction, and various degrees of heating. Preliminary evidence attributes the effect to heat-labile molecules and components residing in the fat fraction. The Milk Against Respiratory Tract Infections and Asthma (MARTHA) trial is currently testing the protective effect of microbiologically safe, minimally processed cow's milk against standard ultra-heat-treated milk in children from 6 months to 3 years with the primary outcome of an asthma diagnosis until age 5 years. If successful, this approach might provide a simple but effective prevention strategy. © 2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;8:878-89)

Key words: Asthma; Allergies; Respiratory infections; Farm exposures; Farm milk; Raw cow's milk; Milk components

INTRODUCTION

The "farm effect" relates to the phenomenon that children growing up on traditionally husbanded farms are less affected by atopic sensitization, asthma, allergic rhinitis, and hay fever compared with children who are raised in rural areas but not on farms.[1-9] Initially observed in central Europe, similar protective effects were found in other parts of Europe, the United States, South America, and New Zealand, pointing toward a global phenomenon.[10-13] In distinct settings, however, increased risk of asthma and wheeze has been described, for example, by exposure to more industrialized farms, hog farming, extensive usage of antibiotics, or in occupational exposure.[14,15] However, the beneficial farm effect observed in children living on traditional farms has been attributed to 2 independent factors, that is, contact to straw and cows, and the consumption of unprocessed cow's milk directly obtained from a farm.[16,17]

Consumption of unprocessed cow's milk is particularly interesting for future preventive measures, because it exerts asthma- and allergy-protective effects also in children otherwise not exposed to farming.[3,18,19] Although the consumption of raw cow's milk bears serious health risk and is strongly discouraged by health authorities, consumption of raw cow's milk and derived raw products including fermented dairy is still common practice in farm families and is often introduced in the children's diet before the first birthday.[13,20] Health and food authorities require raw milk to be heated before consumption, but this cannot be controlled and is often ignored.

In contrast, children not living on farms mainly consume milk from supermarkets ("shop milk"). Some European countries allow the sale of raw milk in special vending machines where it is subjected to strict regulations and permanent controls; however, additional boiling before consumption must be explicitly

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Abbreviations used
ALEX- Allergy and Endotoxin
GABRIEL- Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community
HR- Hazard ratio
microRNA- miRNA
PARSIFAL- Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Life Style
PASTURE- Protection Against Allergy Study in Rural Environments
UHT- Ultra-heat treatment

recommended to consumers. In most countries, pasteurization at 72°C is the minimal requirement for microbial safety.[21] For extended shelf life, heat treatment is performed at high or ultra-high temperature. Besides heat treatment, shop milk usually passes several other processing steps including centrifugation, filtering, and homogenization.

The most profound differences between raw milk and shop milk result from manipulation of the fat fraction and heating. These procedures affect the various components of the complex liquid cow's milk specifically. Moreover, composition and quantity of the components depend on species, feeding, lactation stage, milking frequency, and other environmental factors.[22] Although quantity and functionality of potential health-promoting milk constituents are difficult to disentangle, several candidate molecules have been suggested: whey proteins, microRNA (miRNA), polyunsaturated fatty acids, and oligosaccharides.[18,19,22-30]

In this review, we provide a meta-analysis of all studies worldwide addressing the asthma- and allergy-protective effect of unprocessed cow's milk. In addition, we present an in-depth meta-analysis of the associations between raw cow's milk consumption in early childhood and asthma, current wheeze, atopy, and hay fever in the 4 large Central European studies, whose data were directly available (Allergy and Endotoxin [ALEX] Study; Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Life Style [PARSIFAL]; Protection Against Allergy Study in Rural Environments [PASTURE]; Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community [GABRIEL]). Subsequently, we review the evidence for candidate molecules possibly involved in the protective effect. Finally, we present an approach to demonstrate the farm milk effect in an interventional setting.

META-ANALYSIS OF THE ASTHMA- AND ALLERGY-PROTECTIVE EFFECT OF UNPROCESSED COW'S MILK

The present meta-analysis was conducted in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). A comprehensive literature search was performed within the electronic databases PubMed and ClinicalTrias.gov. The inclusion criteria for the meta-analysis were (1) longitudinal, cross-sectional, or case-control studies, (2) human studies, (3) investigation in the association of cow's milk consumption directly derived from a farm compared with shop milk or no milk consumption and at least 1 of the outcomes of interest (asthma, current asthma/current wheeze, atopy, hay fever), (4) early in life or current consumption of milk reported, (5) age of participants below 20 years, (6) reporting of the odds ratio with CI or *P* value, and (7) publication in English or German. All eligible articles published until June 30, 2019, were included. Meta-analyses, review articles, and case studies were excluded. Definitions of outcomes were based on questionnaire data (standardized International Study of Asthma and Allergies in Childhood [ISAAC] questions) primarily filled by the parents or the participants themselves and skin prick test or specific IgE measurements in the children. In the given age group of schoolchildren, the terms hay fever and seasonal/allergic rhinoconjunctivitis were considered equivalent. Consumption of raw or unprocessed farm milk was defined as reported consumption of either nonpasteurized milk or milk directly derived from a farm without any heating before consumption. The reference group included children either not drinking raw milk or less than once a week (ALEX, PARSIFAL, PASTURE, GABRIEL), or only drinking milk bought in a supermarket, which was at least pasteurized.

Multiple logistic regression models were used to estimate the associations between raw cow's milk consumption and asthma, hay fever, atopy, and eczema. Original study effects were reported as odds ratios and 95% CI or *P* values. Interstudy heterogeneity was studied via Thompson's and Higgins' I^2 criterion. Summary effect sizes were calculated with random-effects models to account for heterogeneity between study effects. DerSimonian-Laird method with inverse variance weighting was used to assign specific weights to the respective studies.

Similarly, an additional meta-analysis was performed using data from the 4 Central European studies (ALEX, PARSIFAL PASTURE, GABRIEL), which were homogeneous with respect to exposure and outcome definitions. Selection of potential confounder variables was based on literature or on the change-in-estimate criterion. Adjusted *P* values of less than .05 were considered significant. All calculations were done with R (R Core Team, Vienna, Austria).[31]

META-ANALYSIS OF ALL STUDIES WORLDWIDE

The database search resulted in 59 matching publications for all 4 outcomes; 12 publications met the inclusion criteria (Figure 1) and reported findings from 8 studies, 6 on early in life and 5 on current milk consumption (Table I). Associations between raw milk consumption in early childhood and asthma, current wheeze, and rhinoconjunctivitis later in childhood were similar across the different studies (Figure 2; $I^2 = 0.0$, 7.6, and 0.0, respectively), but less so for atopy ($I^2 = 45.4$).

Comparability was somewhat limited because periods and definition of exposure, outcome definitions, and study populations differed considerably between the studies. Barnes et al[34] investigated raw milk within the first 5 years, Wickens at al[12] surveyed the first 2 years, whereas the other studies focused on the first year of life. Sozanska et al[33] stratified for frequency of milk consumption and found relevant inverse associations only in children frequently drinking raw milk (Figure 2).

The 4 Central European studies and Perkin and Strachan[32] assessed *current* raw milk consumption and found consistent associations (Figure 3). In PASRIFAL, current raw milk consumption was inversely associated with sensitization to pollen but no other allergen specificities.

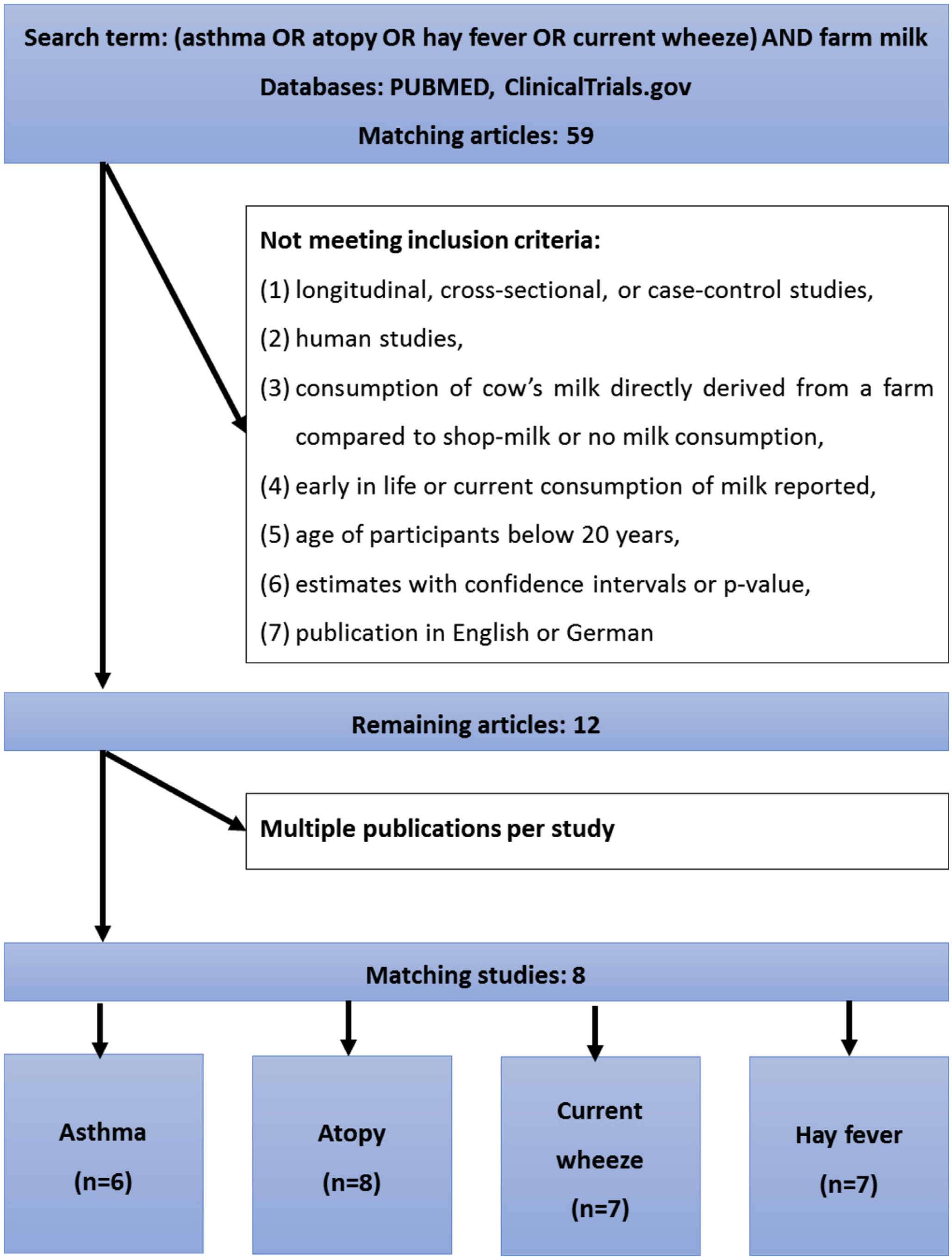

FIGURE 1. Flowchart search strategy.

IN-DEPTH META-ANALYSIS IN THE 4 LARGE CENTRAL EUROPEAN STUDIES

The higher comparability of the 4 Central European studies with respect to study populations, exposure and outcome definitions, and the availability of original data allowed for additional analyses. Stratification according to farming status showed similar effects of early milk consumption on asthma in farm and nonfarm children (Figure 4). Upon stratification, the effects on atopy were weaker in both groups and on hay fever in farm children (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). The effect of current consumption on wheeze was absent in nonfarm children and on atopy in farm children (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org).

In conclusion, we found a consistent protective potential of early and current raw milk consumption for asthma in both farm and nonfarm children and with some limitations for the other outcomes.

TABLE I. Study characteristics

Name/origin	Study population	Exposure: Raw milk consumption	Outcome definition according to ISAAC questionnaire
New Zealand, Wickens et al,[12] 2002	Children (7-10-y-olds) from Dannevirke (New Zealand) and surrounding small towns and rural area (n = 293)	Unpasteurized milk consumption ever vs never in the first 2 y of life (dichotomized: yes vs no)	Asthma: Positive response to ‘‘Has your child ever had asthma?’’
			Wheeze: Positive response to ‘‘Has your child had wheezing or whistling in the chest in the last 12 months?”
			Atopy: Positive SPT result (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, mold mix, cockroach, rye grass, timothy grass, cat, dog)
			AR: Positive response to the question ‘‘Has your child ever had hay fever?’’
The Study of Asthma and Allergy in Shropshire, England, Perkin and Strachan,[32] 2006	Children (5-10-y-olds) from the rural county of Shropshire (n = 4767)	Current unpasteurized milk consumption (dichotomized: yes vs no)	Asthma: Not assessed
	Categorized into 3 strata: children whose families live and work on a farm; children whose families do not live on a farm but whose parents work there; and children without any farm contact (reference group)		Wheeze: Reported current asthma symptoms
			Atopy: Positive SPT result (dog hair, cat hair, horse hair, cow hair, 6-grass mix, house-dust mite [*D pteronyssinus*], *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*)
			AR: Reported current seasonal rhinitis (ISAAC questionnaire)
Poland, Sozanska et al,[33] 2013	Children (5-18-y-olds) from Sobotka and nearby villages in southwest Poland (n = 450). Stratification into 2 strata: children whose families live on a farm and children whose families do not live on a farm (reference group)	Unpasteurized milk consumption (categorized: never, sometimes, regular) in the first year of life	Asthma: Reported doctor’s diagnosis of asthma ever
			Wheeze: Reported current wheeze
			Atopy: Positive SPT result (house-dust mite [*D pteronyssinus*], mixed grass pollens, mixed tree pollens, and cat fur)
			AR: Reported doctor’s diagnosis of hay fever
Crete, Greece, Barnes at al,[34] 2001	Children (11-19-y-olds) from 5 villages in the south of Crete (n = 997)	Consumption of unpasteurized milk straight from the farm in the first 5 y of life (dichotomized: yes vs no)	Asthma: Not assessed
			Wheeze: Not assessed
			Atopy: Positive SPT result (mixed grass pollen [Mediterranean], house-dust mite, cat, Parietaria, olive blossom, Altenaria and goat allergens)
			AR: Not assessed

(continued)

TABLE I. *(Continued)*

Name/origin	Study population	Exposure: Raw milk consumption	Outcome definition according to ISAAC questionnaire
ALEX study, cross-sectional,[3] 1999	Children (6-13-y-olds) from rural areas of Austria, Germany, and Switzerland (n = 2618)	Raw cow's milk directly derived from a farm (consumed at least weekly vs raw cow's milk directly derived from a farm consumed less than weekly, or boiled cow's milk directly bought from a farm or milk bought in a supermarket or no milk consumption at all) in the first year of life and currently (within the last 12 mo before study questionnaire)	Asthma: Reported doctor's diagnosis of asthma or reported doctor's diagnosis of spastic or asthmatic bronchitis at least twice
			Wheeze: Positive response to "In the last 12 months did your child have wheezing or whistling in the chest while breathing?"
			Atopy: Specific IgE (house-dust mite, storage mite, Derp1, timothy grass, cat, cow, hen's egg, cow's milk)
			AR: Reported doctor's diagnosis of hay fever or running nose and itchy eyes in the last 12 mo
PARSIFAL study,[35] cross-sectional, 2006	Children (5-13-y-olds) from Austria, Germany, the Netherlands, Sweden, and Switzerland (n = 15, 137)	Raw cow's milk directly derived from a farm (consumed at least weekly vs raw cow's milk directly derived from a farm consumed less than weekly, or boiled cow's milk directly bought from a farm or milk bought in a supermarket or no milk consumption at all) in the first year of life and currently (within the last 12 mo before study questionnaire)	Asthma: Reported doctor's diagnosis of asthma or reported doctor's diagnosis of obstructive, spastic, or asthmatic bronchitis at least twice
	Categorized into 3 strata: children from farm families; children from anthroposophic families (recruited from Steiner schools); and reference children		Wheeze: Positive response to "In the last 12 months did your child have wheezing or whistling in the chest while breathing?"
			Atopy: Specific IgE (grass pollen-mix, tree pollen-mix, horse, cat, *D pteronyssinus*, *L destructor*)
			AR: Reported doctor's diagnosis of hay fever or running nose and itchy eyes without a cold in the last 12 mo
GABRIEL study,[16] cross-sectional, 3 phases, 2006-2008	Children (6-12-y-olds) from rural areas of southern Germany (Bavaria and Baden-Württemberg), Switzerland, Austria, and Poland (phase I n = 34 491, phase II n = 9668, phase III n = 895) Categorized into 3 strata: children living on a farm run by the family; children not living on a farm but regularly exposed to stables, barns, or cow's milk produced on a farm; and nonexposed nonfarm children	Raw cow's milk directly derived from a farm (consumed at least weekly vs raw cow's milk directly derived from a farm consumed less than weekly, or boiled cow's milk directly bought from a farm or milk bought in a supermarket or no milk consumption at all) in the first year of life and currently (within the last 12 mo before study questionnaire)	Asthma: Doctor's diagnosis of asthma or obstructive bronchitis at least twice
			Wheeze: Positive response to "In the last 12 months did your child have wheezing or whistling in the chest while breathing?"
			Atopy: Specific IgE (*Dermatophagoides pteronyssinus*, cat, rye, timothy grass, birch, mugwort, Phadia gx3)
			AR: Reported doctor's diagnosis of hay fever or running nose and itchy eyes without a cold in the last 12 mo

PASTURE study,[26] birth cohort, started in 2002, follow-up ongoing	Inclusion of pregnant women from rural areas in 5 European countries: Germany, Austria, Switzerland, Finland, and France (n = 1133) Data included from age 1-6 y of the offspring	Raw cow's milk directly derived from a farm consumed at least weekly vs raw cow's milk directly derived from a farm consumed less than weekly, or boiled cow's milk directly bought from a farm or milk bought in a supermarket or no milk consumption at all in the first year of life and currently (within the last 12 mo before study questionnaire)	Asthma: Doctor's diagnosis of asthma or obstructive or spastic bronchitis at least twice
			Wheeze: Positive response to "In the last 12 months did your child have wheezing or whistling in the chest while breathing?" without simultaneously occurring cold
			Atopy: Specific IgE (inhalant allergens)
			AR: Reported doctor's diagnosis of hay fever or allergic rhinitis or sneezing or runny, blocked, or itchy nose without having a cold along with itchy or runny eyes in the last 12 mo

AR, Allergic rhinoconjunctivitis; *ISAAC*, International Study of Asthma and Allergies in Childhood; *SPT*, skin prick test.
Atopy was defined as a positive SPT result (mean wheal diameter of ≥3 mm) or specific IgE level ≥0.35 kU/L.

CANDIDATE MOLECULES POTENTIALLY UNDERLYING THE PROTECTIVE EFFECTS

Cow's milk is a complex lipid-in-water emulsion comprising more than 2000 constituents with 86% to 88% water as its main component.[36,37] Besides the main categories, lipids, proteins, and carbohydrates, there are many other low abundant components, such as vitamins, minerals, and miRNAs.[36,37] Although some of these components are simply dissolved in the water phase, milk also contains many complex structures. For example, the milk contains lipids in the form of droplets (0.1-10 μm) coated with a trilayer of phospholipids and proteins.

Both the milk components themselves and their surrounding structures can be sensitive to heat and pressure. So, they may be altered in quantity or functionality by industrial milk processing and thus be responsible for the protective effect of unprocessed cow's milk. In dairy processing (Table II), milk first undergoes centrifugation to precipitate foreign matter and separate the fat fraction, which allows subsequent adjustment of a specific fat content. After this adjustment of the fat content, milk is generally homogenized to prevent creaming. In this step, the fat globule structure is destroyed and the trilayer of phospholipids and proteins is mostly replaced by milk protein. Subsequently, most shop milk is heated, with the lowest heat treatment generally being pasteurization for inactivation of pathogenic microorganisms. To prolong shelf life and allow uncooled storage, many milks are heated more intensively (eg, extended shelf life or ultra-heat treatment [UHT] milk). Throughout Europe, there are many differences in the heating intensity according to customers' preferences of flavor and storage conditions. Some people prefer the flavor of sterilized over pasteurized milk, whereas others appreciate longer storage duration also at room temperature. In the populations of the 4 Central European studies, there was a strong preference of UHT milk, with a proportion of 30% to 80% of all children drinking predominantly shop milk (see Table E1 in this article's Online Repository at www.jaci-inpractice.org).

Carbohydrates

Carbohydrates are the most abundant constituents in milk, being present at a level of 4.7% to 4.8%. In bovine milk, lactose is by far the most abundant carbohydrate, and present at a very constant level due to its role in the osmotic pressure of milk. Its digestion differs from that of other carbohydrates, and it may serve as a conditional prebiotic.[39] Oligosaccharides are present at much more variable levels. Their prebiotic activity is probably related to the stimulation of the growth of beneficial bacteria in the intestine, which may modulate immune responses and thus protect from asthma and allergies.[40] Likewise, anti-inflammatory mechanisms of oligosaccharides have been described at least for human milk.[41] However, the scientific foundation for effects by carbohydrates from cow's milk is very limited, and the World Allergy Organization recommendations for prebiotic supplementation to prevent asthma and allergic diseases are conditional.[42,43]

Proteins

Proteins are another group of major components accounting for 3% to 4% of the milk.[44] Caseins (80%) are dispersed as a colloidal suspension encased in rather thermostable micelles (100-200 nm). The bioactive whey proteins (20%) are generally present as single globular proteins dissolved in the water phase and undergo profound changes upon heat exposure.[45] Besides α-lactalbumin, β-

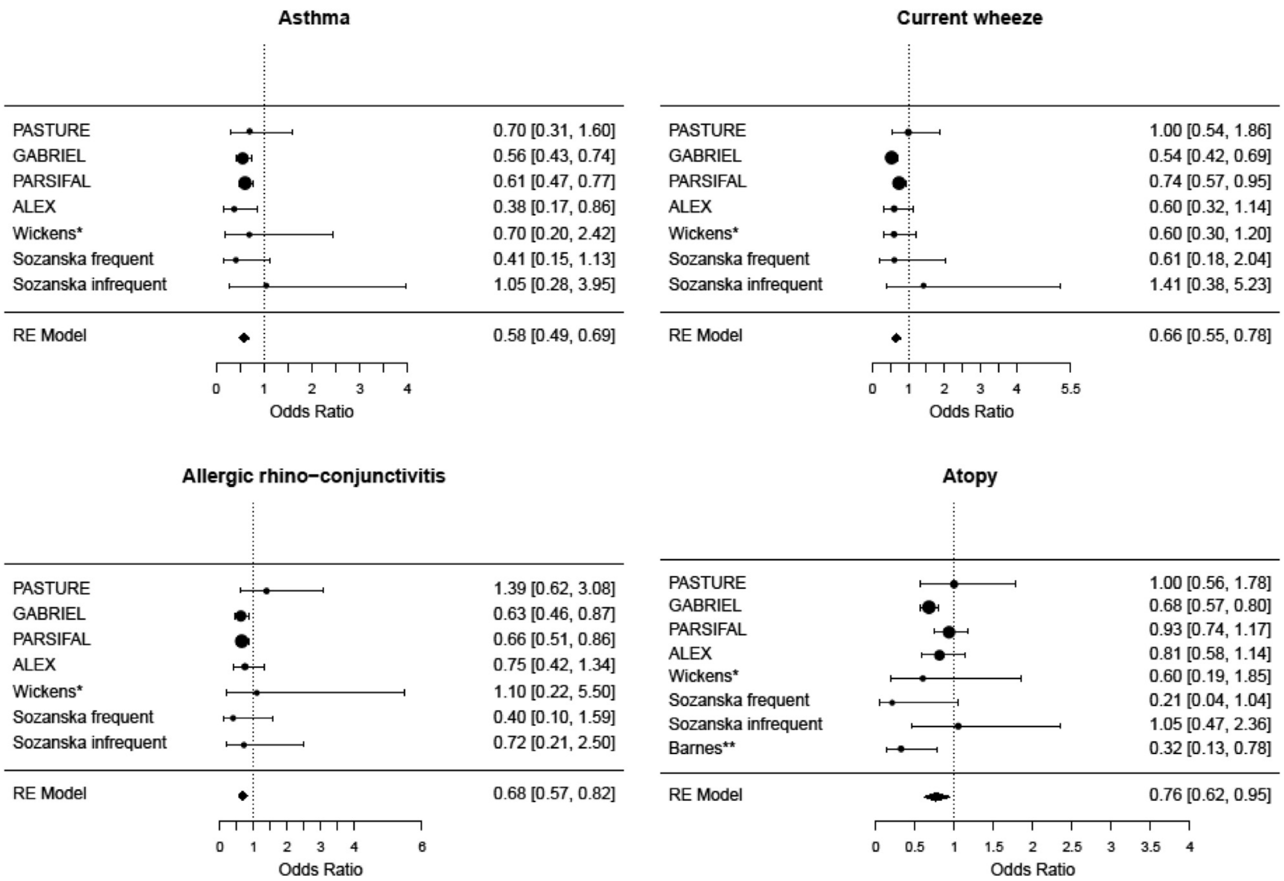

FIGURE 2. Association between raw milk consumption in the first years of life and outcome variables (OR [95% CI]). *OR*, Odds ratio; *RE*, random effects.

lactoglobulin, and the immunoglobulins,[46] low abundant whey proteins such as serum albumin, lactoferrin, lactoperoxidase, and different enzymes and cytokines are hypothesized to play a role in the protective effect.[25] One specific category of protein of interest is the enzymes (eg, alkaline phosphate and lipase), because most of them loose their bioactivity upon heat processing.[44] Although human milk enzymes have been well studied for their health benefits in infants,[47,48] hardly any research is done on the bioactivity of bovine milk enzymes. Generally, proteins are suggested to hold different properties altering development or expression of asthma or allergies, respectively, by modulating the gut microbial composition[49,50] or altering the maturation of the child's immune system toward allergen tolerance and thus reduce inflammatory reactions.[33,51,52]

Fatty acids

Of the main milk components, the lipid fraction is the most variable, because it can be affected by feeding, lactation status, animal breed, and season. In commercially available shop milk, the fat fraction is altered by several industrially applied processing steps. The fat content of unprocessed cow's milk naturally ranges from 3% to 6%,[37] whereas commercially available milk is generally standardized to a fat content of, for example, 3.5% or 1.5%. The fat globules are coated by a trilayer of membrane; these structures contain 400 different fatty acids and mono-, di-, and triglycerides, phospholipids, cholesterol, fat-soluble vitamins, and hundreds of different proteins. During homogenization, the structure of these fat globules is broken under high pressure to create smaller fat globules, aiming to prevent fat creaming in the final dairy product.[25] This alteration of both the fat content and the fat globule structure might contribute to the loss of the health-promoting effect of processed cow's milk as exemplified for milk fat globule membranes.[53] Because both homogenization and heating affect the composition of this membrane material, these beneficial effects may be lost upon industrial processing.[54,55]

With respect to quantitative fat content, the PARSIFAL study revealed a reduced asthma risk in children consuming full-cream milk or farm-produced butter.[35] In the PASTURE study, an asthma-protective effect of cow's milk holding a higher fat content emerged. This fat effect was attributable to higher n-3 polyunsaturated fatty acid levels and a lower n-6/n-3 (polyunsaturated fatty acid) ratio in raw cow's milk as compared with industrially processed milk.[26] Moreover, higher contents of short-chain fatty acids in milk and milk products tended to be associated with a lower prevalence of asthma, atopic sensitization, food allergy, and allergic rhinitis.[56] In addition, other milk constituents such as enzymes stored in the milk fat globules[57] might be diminished by reduction of the fat content of shop milk or their functionality might be altered under high-pressure treatment during homogenization.

Minerals, vitamins, and hormones

Milk meets all physiologic needs of a neonate and provides a large variety of micronutrients.[44] Because of their chemical properties, minerals are not influenced by the industrial

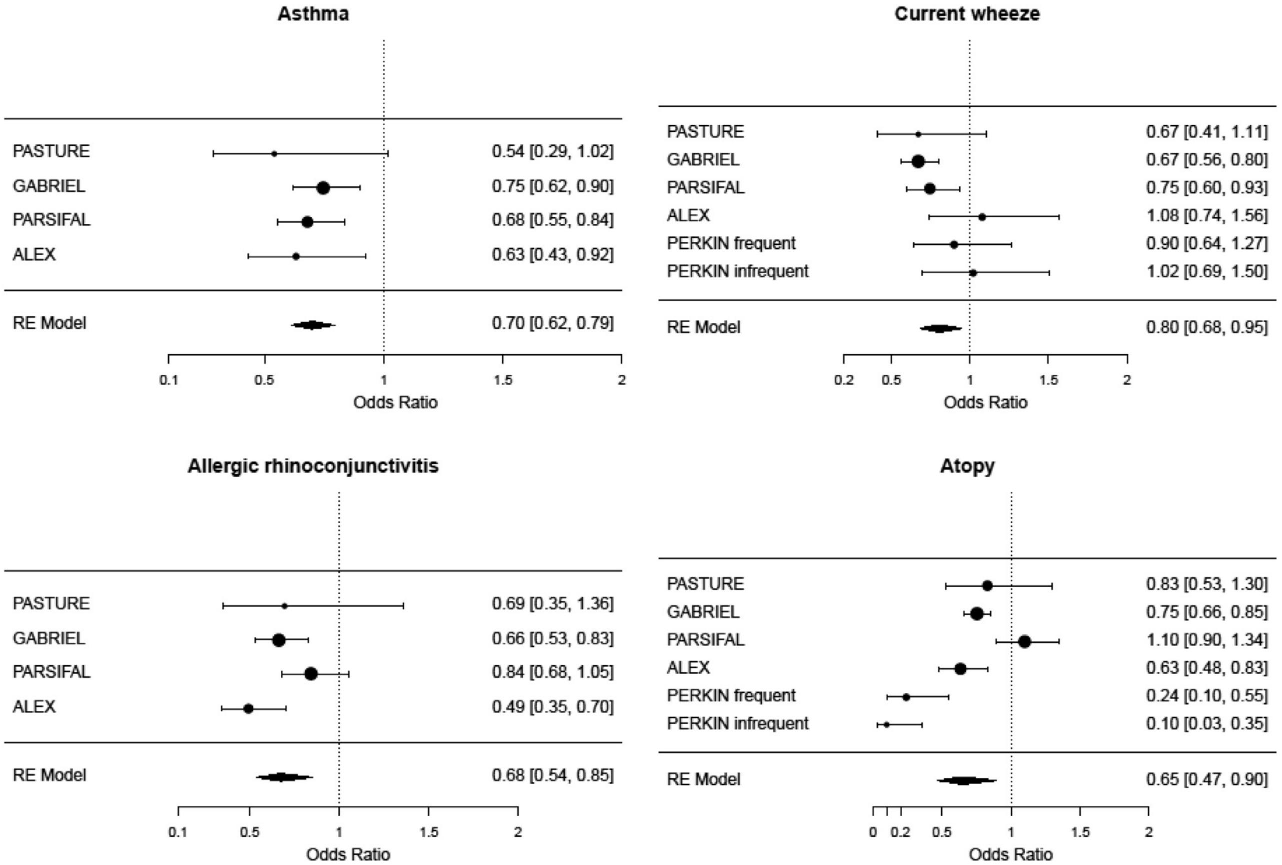

FIGURE 3. Association between current raw milk consumption and outcome variables (OR [95% CI]). *OR*, Odds ratio; *RE*, random effects.

processing. In contrast, many vitamins are heat-labile. Pasteurization generally does not cause a loss of vitamins,[58] whereas UHT sterilization and the resulting longer storage duration cause a limited loss of several vitamins.[59,60] However, the potential immunologic consequences of the loss of these vitamins upon UHT sterilization have not been studied.

Milk is a source of many different hormones, including among others growth factors and steroid and reproductive hormones.[61,62]

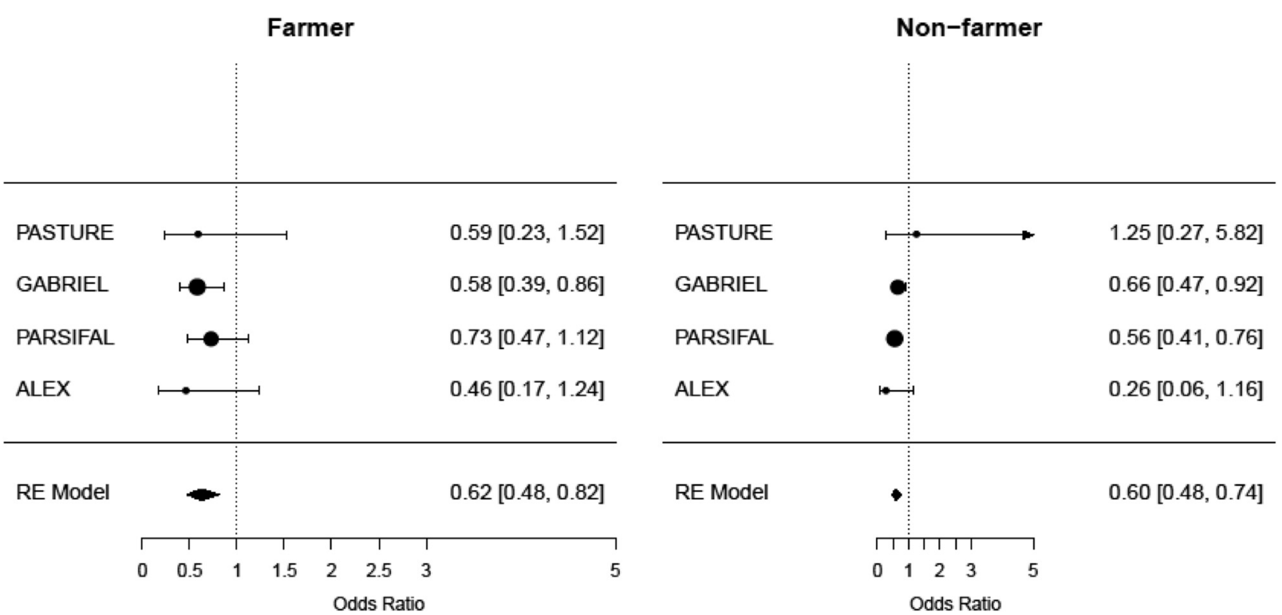

FIGURE 4. Raw cow's milk consumption in the first year of life and childhood asthma (OR [95% CI]). *OR*, Odds ratio; *RE*, random effects.

TABLE II. Industrially applied milk processing steps

Processing step	Comments
1. Centrifugation	Separation of dirt particles, somatic cells, and cream at 50°C
2. Adjustment of milk fat content	Commercially available milk is offered in 4 different categories:
	-natural full-cream milk (>3.5% fat)
	-full-cream milk (3.5% fat)
	-semiskimmed milk (1.5%-1.8% fat)
	-skimmed milk (≤0.3% fat)
3. Heat treatment (to kill potential pathogens and prolong shelf life)	
a. Pasteurization	Heating (72°C-75°C) for 20-30 s
b. High-heat treatment (extended shelf life)	Preheating at 95°C for 20 s, direct steam injection at 127°C for 5 s
c. UHT milk	Preheating at 93°C for 23 s, direct steam injection at 142°C for 5 s
d. Sterilization	Milk bottling, heating at 110°C -120°C for 10-30 min
4. Homogenization	2-stage homogenization* at 250/50 bar

Processing steps are listed in the order they are usually applied in dairy companies in Bavaria[28,38]; this order might slightly vary over countries.
*Milk is pressed through fine nozzles to reduce fat globule sizes and prevent creaming.

The heat sensitivity differs largely between individual hormones, from complete to no inactivation by industrial processing.[63-65] Health effects of milk-derived hormones are known,[66] but effects on the immune development have hardly been studied, and not all in the context of allergy and asthma.[62] However, knowing that levels of hormones derived from dairy product intake are relatively small compared with endogenous hormone production and the hormone levels in breast milk, a major role of bovine milk—derived hormone intake is not to be expected.[65]

microRNA

With recent advances in analytical methodology such as high-throughput sequencing, new milk components have been detected, among them miRNA, which are short noncoding RNA sequences. Cow's milk miRNAs strongly resemble human milk miRNAs and might thus be able to attach to human mRNA and thereby affect gene expression posttranscriptionally by regulating mRNA degradation and translation initiation at ribosomes. The possible effects on the human immune system are not yet clear; however, a reduced susceptibility to the development of asthma and allergies has been proposed.[28,67-69] miRNA species interfering with asthma genes were found to be affected by heat treatment as performed during industrial processing.[28] In milk samples of the GABRIEL study, we found substantial differences between total miRNA quantities in farm and shop milk, albeit not directly related to asthma status (Figure 5; see Table E2 in this article's Online Repository at www.jaci-inpractice.org). Furthermore, we detected specific miRNA levels at consistently lower levels in UHT milk as compared with raw milk (eg, miR_21_5p hazard ratio [HR] = 0.40, $P < .001$ in GABRIEL and HR = 0.15, $P < .001$ in PASTURE). Again, this discrepancy did not explain the protective effect of farm milk. For example, the significant association of miR_148a_3p with asthma status in GABRIEL (HR = 0.44; $P = .024$) was changed by adjustment for milk type to HR = 0.85 ($P = .667$) by about 80% and thereby largely explained. Consequently, miRNAs are less likely to carry a substantial share of the asthma-protective effect. In practical terms, however, they may serve as a proxy for the quality and the asthma-protective potential of a milk type.

Cellular structures

Besides the milk components, milk contains cells and cellular fragments of different origin. Exosomes, that is, extracellular vesicles, secreted among others by mammary gland epithelial cells, can transport different components, including proteins and miRNAs.[70,71] With these contents, milk-derived exosomes may affect the immune development of infants and their risk of allergy,[72,73] particularly because the exosomes and their cargo may be absorbed intestinally.[74] The effect of processing on exosomes as such has not been studied, but it has been shown that the miRNAs contained in exosomes are largely degraded upon heating similarly to miRNA itself.[75]

Milk also contains bovine somatic cells, predominantly leukocytes. The composition of the somatic cell fraction depends largely on the health status of the cow.[76] Animal studies suggest that somatic cells in unprocessed milk may be absorbed by the suckling neonate.[77] During industrial dairy processing, these somatic cells are generally removed by the initial centrifugation step.[78] Although their impact on human health has not been studied, xenogeneic pressure on the developing immune system is conceivable.[79]

An obvious difference between raw cow's milk and heat-treated shop milk is the microbial contamination. Heat treatment to at least pasteurization level (Table II) is essential to inactivate potential pathogens such as *Escherichia coli* or *Staphylococci*, thereby ensuring physiologically safe milk consumption.[80] Because pathogenic microorganisms are not selectively inactivated by heat or removed by centrifugation, total bacterial counts are substantially lower in industrially processed milk.[19,81] Involvement of microorganisms in the beneficial effect of raw milk seems obvious,[18,82] though the underlying mechanisms are not completely understood. Possibly bacterial endotoxin might induce tolerance toward allergens.[25,83-85] Moreover, microbiota may operate as probiotics and thus indirectly shape the microbial colonization of the gut early in infancy. Varying with study region, 30% to 50% of the children receive cow's milk, particularly farm milk within the first year of life.[20,86] During this period, the gut microbiome is primarily influenced by dietary factors, which may subsequently affect health conditions such as asthma and allergies.[22,87-90]

MILK CONSUMPTION HABITS

Besides processing differences, consumption habits might differ between children drinking raw cow's milk and those drinking shop milk, particularly with respect to storage time and

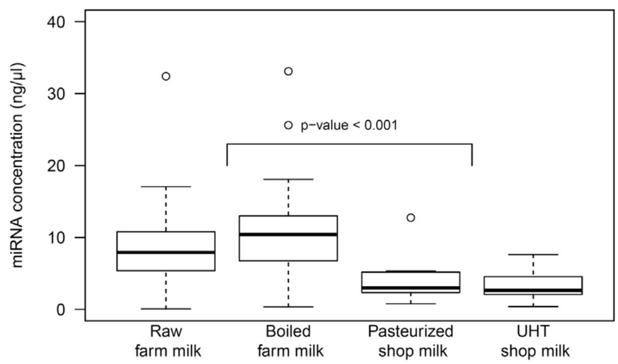

FIGURE 5. Concentration of miRNA in milk types.

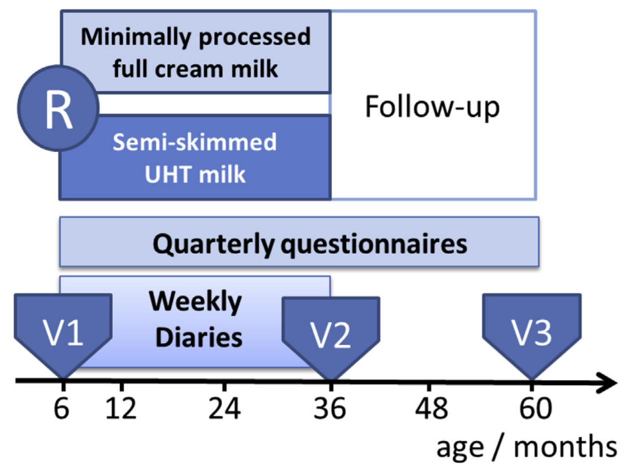

FIGURE 6. Design of the MARTHA trial. *R*, Randomization; *V*, clinical visit.

quantity of consumed milk. In GABRIEL and PASTURE, raw milk was mainly consumed within the day of milking (see Table E3 in this article's Online Repository at www.jaci-inpractice.org), whereas shop milk is often stored for 2 or more days between opening and consumption. As described above for vitamins, the levels of labile ingredients might decrease during prolonged storage, particularly in UHT milk. Because milk is a nutritious environment, heat-resistant microorganisms might proliferate and disintegrate health-promoting milk constituents.[91]

The frequency and overall amount of milk consumed by farm and shop milk drinkers might also vary. Among children drinking milk at least weekly, daily consumption was 20% more common for farm milk as compared with shop milk (see Table E4 in this article's Online Repository at www.jaci-inpractice.org). An unpublished analysis of a food frequency questionnaire from the ALEX study revealed that farm children consumed 207 g milk or milk products daily, whereas nonfarm children consumed on average 172 g/d ($P < .1$) corresponding to a means ratio of 1.20 (95% CI, 1.00-1.44; $P < .1$). In conclusion, quantitative differences in milk consumption may moderately contribute to the beneficial effect of raw milk on asthma and allergies.

INTERVENTION STUDY ASSESSING MINIMALLY PROCESSED MILK

As detailed above, we are far from understanding what actually drives the beneficial raw milk effect. Observational studies are limited in the range of the exposure; that is, there is no continuum of milk ingredients over the milk types. UHT milk, for example, has low levels of intact whey proteins, miRNA, vitamins, and other heat-susceptible components, which makes it nearly impossible to disentangle the health effects of the respective ingredient. Moreover, observational studies are hampered by the notorious difficulties with confounding and information bias.

A pragmatic and more promising approach would consist in an experimental setting directly comparing the effects of raw farm milk against shop milk. The low but existent risk of life-threatening infections, however, precludes any testing of raw milk in humans. The ideal milk for such a trial would be microbiologically safe but otherwise not exposed to any industrial processing.

The ongoing Milk Against Respiratory Tract Infections and Asthma (MARTHA) trial[92] now fills this gap. Supported by Dutch Longfonds, the University Children's Hospitals Munich and Regensburg have already started recruitment. The study has been set up to compare a minimally processed, safe full-cream milk against semiskimmed UHT milk. The latter was chosen as a comparator because it reflects "standard care": it is very common in infant nutrition and the basis for most infant formulas. Children receive a daily dose of 200 mL and, from the 11th month, 2 × 150 mL, conforming with national recommendations on nutrition of babies and infants.[93] Additional nutrition is ad libitum, which compensates for slight differences in energy value between the milk preparations. Irrespective of the family history of atopy, children are recruited and randomized to 1 of the 2 arms between age 6 and 12 months (Figure 6). The intervention starts as soon as children are no longer fully breast-fed and lasts until the third birthday. Thus, the intervention covers a period when cow's milk consumption is very common and has been shown to affect various health outcomes.[26] Physicians examine the children at inclusion, after intervention at age 3 years, and after follow-up at age 5 years. Parents complete weekly diaries for assessment of respiratory health and symptoms suggestive of adverse outcomes such as cow's milk allergy, and lactose or milk protein intolerance. The primary outcome "asthma as diagnosed by a physician" will be assessed at 5 years, which explains the long duration of the trial. Secondary outcomes are respiratory infections and wheeze during the intervention, low-grade inflammation, atopic sensitization, and eczema at 3 and 5 years. The MARTHA trial is registered with the German trial registry as DRKS00014781,[94] where more details on the trial can be found.

OUTLOOK

Regular consumption of minimally processed milk instead of industrially processed milk with long shelf life would be an attractive prevention because it is simple, acceptable, and easy to implement without major changes in lifestyle. From a scientific point of view, however, further research into the underlying mechanisms of the farm milk effect would be highly desirable.

Subsequent trials might assess the protective potential of the various components of cow's milk. Understanding the prevention might also foster understanding of the disease. Nevertheless, the example of John Snow's successful fight against the cholera epidemic in 19th-century London[95] teaches us that interventions can be effective even in the absence of a valid theory about pathomechanisms.

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ONLINE REPOSITORY

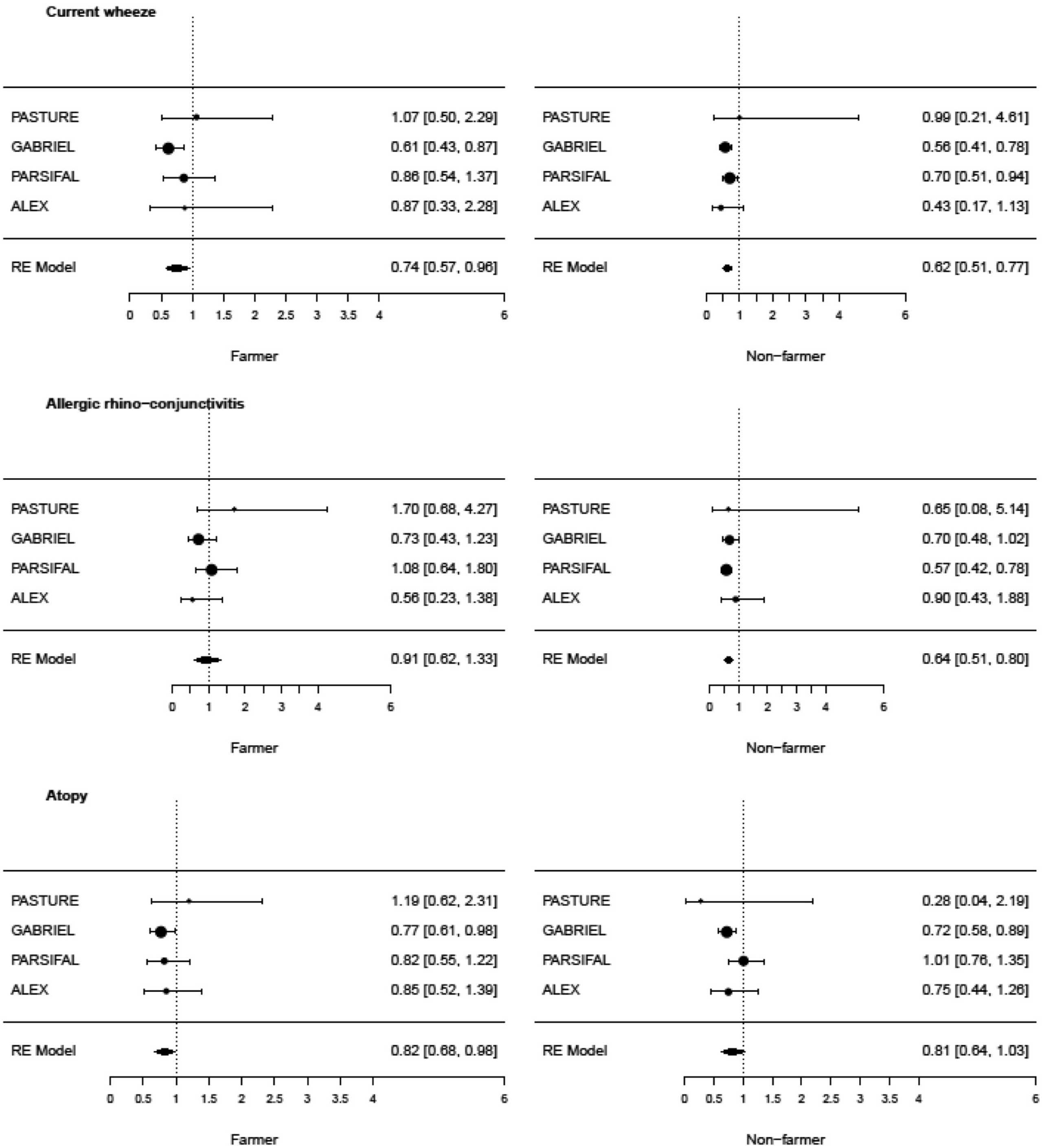

FIGURE E1. Raw cow's milk consumption in the first year of life and outcome variables. *RE*, Random effects.

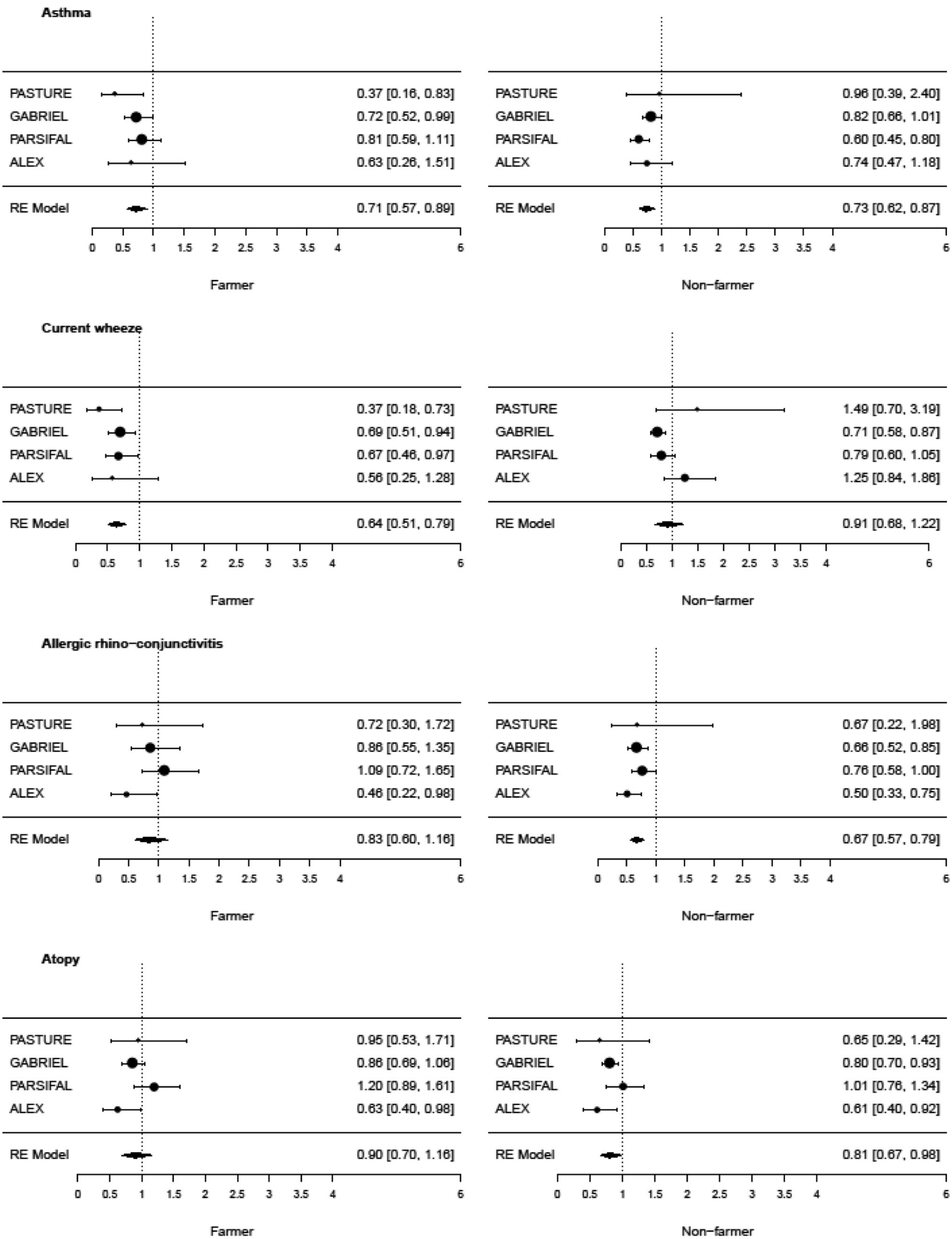

FIGURE E2. Current raw cow's milk consumption and outcome variables. *RE*, Random effects.

TABLE E1. Proportion of children drinking UHT milk among those drinking shop milk

Study	% of children drinking UHT milk*
GABRIEL	77.4% (429 of 554 children)
PASTURE	53.7% (267 of 497 children)
ALEX	44.8% (490 of 1093 children)
PARSIFAL	28.5% (2514 of 8816 children)

*Among those with information on specific milk type.

TABLE E2. Associations between total miRNA levels and milk type (linear regression)

Milk type	β estimate	SE	*P* value
Intercept	28.38		
Pasteurized vs raw cow's milk	1.87	0.58	.001
Boiled vs raw cow's milk	2.30	0.40	<.001
UHT vs raw cow's milk	5.29	0.39	<.001

TABLE E3. Proportion of milks consumed within 1 d after milking or after opening the milk bottle, respectively

Study	Age of assessment (y)	Raw farm milk	Shop milk
GABRIEL	6-12	55%	12%
PASTURE	6	94%	37%

TABLE E4. Daily consumption within children consuming milk at least weekly

Study	Age of assessment (y)	Farm milk	Shop milk	Ratio
GABRIEL	6-12	68%	56%	1.20
PASTURE	6	72%	60%	1.20