Continuous Rather Than Solely Early Farm Exposure Protects From Hay Fever Development

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Original Article

# Continuous Rather Than Solely Early Farm Exposure Protects From Hay Fever Development

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What is already known about this topic? The protective effects of early-life farm exposures and gut microbiome composition on atopic diseases and asthma propose an important window of opportunity.

What does this article add to our knowledge? Early-life farm exposures also determine the risk of hay fever. However, continuous farm milk consumption is necessary for optimal prevention, thereby arguing against the notion of an early-determined trajectory governing later outcomes.

How does this study impact current management guidelines? These results emphasize the preventive potential of continuously drinking unprocessed farm milk for hay fever protection, suggesting carrying out clinical trials to test microbiologically safe cow's milk for protection from hay fever.

VISUAL SUMMARY

Continuous consumption of farm milk ----→ Potentially mediated by gut microbiome ----→ **Protection of hay fever**
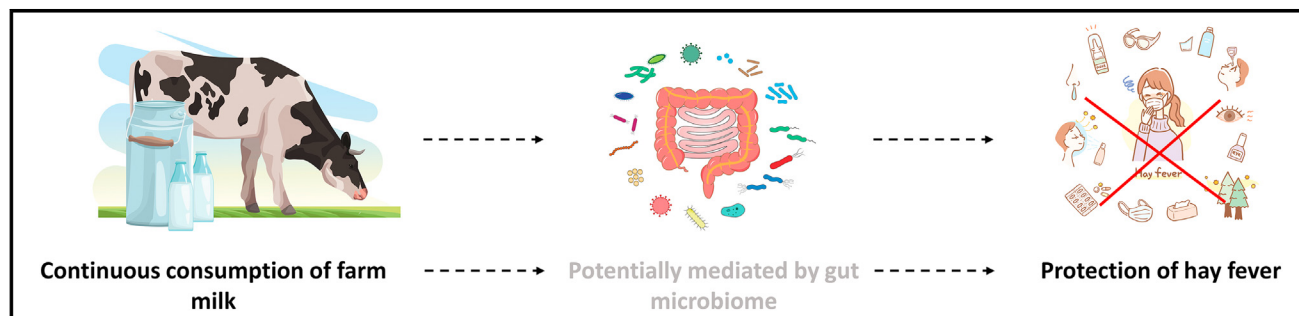
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BACKGROUND: An important window of opportunity for early-life exposures has been proposed for the development of atopic eczema and asthma.

OBJECTIVE: However, it is unknown whether hay fever with a peak incidence around late school age to adolescence is similarly determined very early in life.

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Abbreviations used
aOR- Adjusted odds ratio
GMR- Geometric mean ratio
NNT- Number needed to treat
PASTURE- Protection Against Allergy Study in Rural Environments
RMLCA- Repeated measure latent class analyses
SCFAs- Short-chain fatty acids
SPT- Skin prick test

METHODS: In the Protection against Allergy-Study in Rural Environments (PASTURE) birth cohort potentially relevant exposures such as farm milk consumption and exposure to animal sheds were assessed at multiple time points from infancy to age 10.5 years and classified by repeated measure latent class analyses (n = 769). Fecal samples at ages 2 and 12 months were sequenced by 16S rRNA. Hay fever was defined by parent-reported symptoms and/or physician's diagnosis of hay fever in the last 12 months using questionnaires at 10.5 years.

RESULTS: Farm children had half the risk of hay fever at 10.5 years (adjusted odds ratio [aOR] 0.50; 95% CI 0.31−0.79) than that of nonfarm children. Whereas early life events such as gut microbiome richness at 12 months (aOR 0.66; 95% CI 0.46−0.96) and exposure to animal sheds in the first 3 years of life (aOR 0.26; 95% CI 0.06−1.15) were determinants of hay fever, the continuous consumption of farm milk from infancy up to school age was necessary to exert the protective effect (aOR 0.35; 95% CI 0.17−0.72).

CONCLUSIONS: While early life events determine the risk of subsequent hay fever, continuous exposure is necessary to achieve protection. These findings argue against the notion that only early life exposures set long-lasting trajectories. © 2022 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/

INTRODUCTION

Hay fever is the most common allergic disease worldwide with prevalence between 20% and 30%.[1] The high prevalence has a vast impact on several factors such as quality of life and high health care costs.[2,3] Numerous epidemiological studies have shown the protective effect of early-life farm exposures and gut microbiome composition on asthma, atopy, atopic sensitization, and hay fever[4-11]; thus, proposing an important window of opportunity for early-life farm exposures and gut microbiome composition for the protection of atopic diseases and asthma. However, it is unknown whether hay fever with a peak incidence around late school age to adolescence is only determined very early in life or whether later exposure before the onset of disease matters most.

The protective farm effect has been attributed to 2 factors; consumption of unprocessed cow's milk, subsequently termed farm milk, and exposure to animal sheds.[12-16] Hence, the aim of these analyses is to study the temporal pattern of these protective exposures on hay fever development using the longitudinal data from the Protection Against Allergy Study in Rural Environments (PASTURE) study. Furthermore, the role of the gut microbiome was investigated.

METHODS
Study design and population

The PASTURE study is a prospective birth cohort study started in 2002 and is conducted in children from rural areas of 5 European countries (Austria, Finland, France, Germany, and Switzerland).[17] The study was designed to evaluate risk and preventive factors for atopic diseases. The study was approved by the local research ethics committees in each country, and written informed consents were obtained from the children's parents. Pregnant women were invited to participate during their third trimester of pregnancy. The children from the participating women were recruited at birth. Children of mothers living on family-run livestock farms at birth of the children were assigned to the farm group. The nonfarm group included children of mothers from the same rural areas but not living on a farm.[18] Information was obtained through questionnaires in interviews or self-administered questionnaires from mothers.

Definitions of outcome. Hay fever was defined by parent-reported symptoms (itchy, runny, or blocked nose without a cold accompanied by red itchy eyes) and/or a physician's diagnosis of hay fever in the last 12 months using questionnaires at 10.5 years. Allergen-specific immunoglobulin E and skin prick test (SPT) were assessed at 10.5 years.[19] Inhalant sensitization was defined as at least 1 immunoglobulin E specific to alder, birch, hazel, plantain, mugwort, alternaria, grass, rye, *Dermatophagoides(D.) pteronyssinus, D. farina,* cat, dog, or horse at levels of 0.7 I UmL^{-1} or greater or SPT (birch, grass, alternaria, *D. pteronyssinus, D. farinae,* cat, or dog) of 3 mm or greater. A more stringent definition of hay fever consisting of hay fever plus inhalant sensitization at 10.5 years was used in sensitivity analyses.

Assessment of exposures. The child's consumption of any farm milk, pasteurized, and homogenized milk subsequently termed processed milk consumption, and any exposure to animal sheds (cows, pigs, sheep, or horses) at time points 12 and 18 months and 2, 3, 4, 5, 6, and 10.5 years were assessed. In addition, maternal any farm milk consumption and animal sheds exposure was assessed during pregnancy and infant's consumption of any farm milk, processed milk, and exposure to animal sheds (4−12 months) were obtained on a weekly basis by diary. The exposure to animal sheds was further dichotomized based on the third quartile (17 weeks) weeks spent on animal sheds as a cut-off.

Avoidance of milk or milk products was assessed at 12 and 18 months and 2, 3, 4, 5, and 6 years. In addition, information on the frequency of farm milk consumption was assessed at 18 months and 2, 3, 4, 5, 6 and 10.5 years. The frequency of processed milk consumption was assessed at 10.5 years.

FIGURE 1. Types of exposure classes. Solution for repeated measure latent classes defined by different exposures, which are (**A**) exposure to animal sheds and (**B**) farm milk consumption in the PASTURE children. Numbers in parentheses indicate the total number of children in each class.

DNA extraction from fecal samples and sequencing analyses. Fecal samples were collected from children's diapers during the home visit at 2 and 12 months. The DNA was extracted from homogenized samples and bioinformatics processing were performed as previously described in detail.[10] Briefly, α-diversity (ie, richness and Shannon index) was calculated as the average of multiple times rarefied samples.[10] Metabolite levels of short-chain fatty acids (SCFA) were measured in fecal samples obtained from 301 children at 12 months.[20,21] Two variables, butyrate and propionate scores, were created by modeling SCFA levels on the relative abundance of all bacterial genera using random forest model in the R-package ranger.

Statistical analyses

We performed repeated measure latent class analyses (RMLCA) using data from pregnancy to 10.5 years (ie, 9 time points) were included separately for exposure to animal sheds and farm milk consumption (Figure 1). The children were allocated to specific exposure classes by their highest posterior probabilities. The analyses were done on children having data at least at 7 of the 9 assessed time points. The optimal number of exposure classes was then determined according to the Bayesian Information Criterion, and the labelling of the exposure classes was based on main features of each class.

Further as sensitivity analyses, we repeated the farm milk RMLCA in the subgroup of children without a family history of parental asthma and/or atopy and excluding children avoiding milk or milk products at 1 to 6 years as it could introduce confounding by reverse causation (ie, a positive family history). A farm milk consumption score (see Methods; available in this article's Online Repository at www.jaci-inpractice.org) reflecting the frequency of farm milk consumed was built and divided into quintiles. The quintiles were further categorized as low (q1), intermediate (q2–q4), and high (q5).

The associations between hay fever and potential exposures (farm milk exposure classes, animal sheds exposure classes, frequency of farm milk consumption [continuous and quintiles], frequency of processed milk consumption, SCFAs [butyrate score and propionate score] as well as gut microbiome's richness, and Shannon index) were assessed by logistic regression. We tested the differences in relative abundance of the most common single bacterial genera at 2 and 12 months with hay fever by the Wilcoxon test.[10] The associations between gut microbiome richness and farm milk

TABLE I. Description of the study population*

Characteristic	All (n = 769) n (%)/Total	Hay fever (n = 99 [12.9%]) n (%)/Total	No hay fever (n = 670 [87.1%]) n (%)/Total	P value
Farm child (yes)†	367 (47.7)/768	31 (31.3)/99	336 (50.2)/670	.0005
Exposure to cats at 2 mo (yes)‡	199 (26.0)/767	19 (19.2)/99	180 (27.0)/668	.11
Exposure to dogs at 2 mo (yes)‡	147 (19.2)/766	17 (17.2)/99	130 (19.5)/667	.68
Maternal age at pregnancy (y), mean ± SD	31.2 ± 4.5 (n = 769)	31.4 ± 4.4 (n = 99)	31.2 ± 4.5 (n = 670)	.52
Maternal smoking (yes)§	96 (12.5)/766	16 (16.5)/97	80 (12.0)/669	.25
Second-hand smoking (yes)‖	33 (4.3)/764	3 (3.1)/98	30 (4.5)/666	.79
Parental education (yes)¶				.13
Low	62 (8.1)/764	3 (3.1)/97	59 (8.9)/667	
Medium	280 (36.7/764)	39 (40.2)/97	241 (36.1)/667	
High	422 (56.7)/764	55 (56.7)/97	367 (55.0)/667	
Use of antibiotics during pregnancy (yes)#	204 (27.0)/755	26 (26.5)/98	178 (27.1)/657	.99
Parental atopy (yes)**	416 (54.4)/765	72 (73.5)/98	344 (51.6)/667	<.0001
Mode of delivery (normal)	624 (81.9)/762	82 (83.7)/98	542 (81.6)/664	.68
Premature birth (yes)††	11 (1.4)/769	1 (1.0)/99	10 (1.5)/670	.99
Birth weight (kg), mean ± SD	3.4 ± 0.44 (n = 605)	3.4 ± 0.5 (n = 82)	3.4 ± 0.4 (n = 523)	.81
Breastfeeding 2 mo (yes)‡‡	711 (92.7)/767	90 (90.9)/99	621 (93.0)/668	.41
Gender (female)	366 (47.7)/768	42 (42.4)/99	324 (48.4)/669	.28
Having siblings (yes)	494 (64.2)/769	60 (60.6)/99	434 (64.8)/670	.43
Use of antibiotics during first y of life (wk), mean ± SD§§	0.03 ± 0.3 (n = 746)	0.01 ± 0.1 (n = 97)	0.03 ± 0.4 (n = 649)	.86
Doctor's diagnosis of hay fever (yes)	36 (4.7)/769	36 (36.4)/99	NA	NA
Inhalant sensitization (IgE ≥ 0.7 kU/L or SPT ≥ 3 mm) at 10.5 y‖‖	259 (49.6)/522	66 (86.8)/76¶¶	193 (43.3)/446¶¶	<.0001
Concomitants##				
Asthma (yes)	69 (9.0)/764	28 (28.9)/97	41 (6.2)/667	<.0001
Eczema (yes)	100 (13.1)/763	36 (36.7)/98	64 (9.6)/665	<.0001
Food allergy (yes)	41 (5.5)/746	21 (21.7)/97	20 (3.1) /649	<.0001

IgE, Immunoglobulin E; *NA,* not applicable.

*The categorical variables are presented as frequency (percentage) and the continuous variables as mean. The tests for differences between the groups are χ^2 or Fischer exact test for categorical variables and Mann-Whitney U test for continuous variables.

†Farm child was defined as "Children of mothers living on family-run livestock farms were assigned to the farm group. The nonfarm group included children of mothers from the same rural areas but not living on a farm.

‡Exposure to pets at 2 mo (cats and dogs) was defined by asking "If you have cats?," "If you have dogs?," and "If they stay indoors in the house?"

§Maternal smoking during pregnancy was defined using the following questions, "Have you in your life smoked more than 5 packs of cigarettes?" Or "Have you quit smoking in the meantime?" and if yes, "Was it during this pregnancy?" Smoking by father, "Have you in your life smoked more than 5 packs of cigarettes?" Or "Do you still smoke?"

‖Second-hand smoking "How many cigarettes are on average per day were smoked in your house by other people?" If > 1 then second-hand smoking was defined as 1; else 0.

¶Parental education was defined as low (<10 y), medium (10 y) and high (>10 y).

#Use of antibiotics during pregnancy was defined by asking, "Have you taken antibiotics since the beginning of pregnancy?" Or "Have you taken any antibiotics during this pregnancy?"

**Parental atopy was defined as doctor's diagnosis of hay fever, atopic dermatitis, or asthma ever in mother or father.

††Child was defined as premature if the child was born before the completion of 37 wk of pregnancy.

‡‡Breastfeeding at 2 months (yes or no) was defined by asking, "If you have ever breastfed?"

§§Use of antibiotics by a child during the first year of life was defined as, "Total no. of weeks with antibiotics ingested."

‖‖Inhalant sensitization was defined as at least 1 IgE specific to alder, birch, hazel, plantain, mugwort, alternaria, grass, rye, *Dermatophagoides pteronyssinus, D, farina,* cat, dog, or horse at levels ≥ 0.7I UmL^{-1} or SPT (birch, grass, alternaria, *D. pteronyssinus, D. farinae,* cat, or dog) ≥ 3mm. Serum-specific IgE and SPT was not measured in the Austrian study center; hence, only subsample n = 522 was included.

##Asthma was defined as a physician's diagnosis of asthma or recurrent obstructive bronchitis established until 10.5 y. Eczema and food allergy were defined as physician diagnoses at least once until 10.5 y.

consumption, processed milk consumption, and exposure to animal sheds during infancy was assessed by linear regression. The effect estimates are presented as adjusted odds ratios (aORs) for logistic regression and geometric mean ratios (GMRs; calculated by exponentiation of the regression coefficients and their 95% CIs) for linear regression along with their respective 95% CI and a P value of .05 was considered significant. The previously listed models were adjusted for centers and confounders (growing up on a farm and

parental asthma and/or atopy) associated with hay fever and exposures in our study. No other confounders (ie, associated with both outcome and exposures) were found. In addition, we calculated the number needed to treat (NNT), which is the effectiveness of a treatment on an outcome using an R-script.[22]

Furthermore, we conducted mediation analyses to assess whether the associations between farm milk consumption and exposure to animal sheds in infancy (4–12 months) and the risk of hay fever is
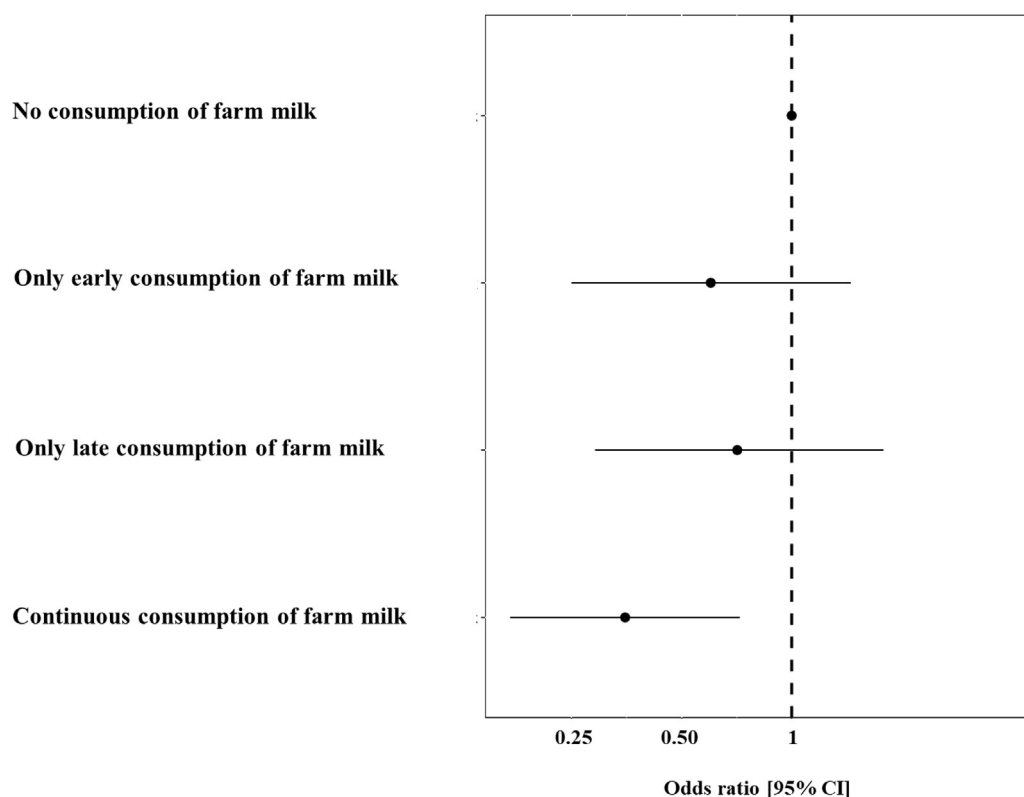
FIGURE 2. Associations of farm milk exposure classes with hay fever at 10.5 years. Models are adjusted for centers, growing up on a farm, and parental atopy. The forest plot represents the aOR with 95% CI.

mediated by gut microbiome features adjusting for centers. The mediation analysis was conducted through path analysis using the maximum likelihood test to estimate the regression parameters in Mplus 8.5.[23] The mediating effect is reported as the proportion of the estimated indirect effect to the total effect.

The statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC) and Mplus 8.5 software (Muthén & Muthén, Los Angeles, CA).

RESULTS
Characteristics of the study population

At 10.5-year follow-up, 778 children participated in the PASTURE study and 769 have data on hay fever. Comparing the baseline characteristics between included (n = 769) and excluded children (n = 364) did not show any significant difference except for maternal age at pregnancy, maternal smoking, parental education, and premature birth (Table E1; available in this article's Online Repository at www.jaci-inpractice.org). Data on farm milk consumption and exposure to animal sheds at least at 1 time point (from pregnancy, 12 and 18 months, and 2, 3, 4, 5, 6, and 10.5 years) were available for all these children. Of these, 769 children had information on hay fever at 10.5 years. The proportion of children growing up on a farm was 47.7%. Hay fever at 10.5 years was reported in 12.9% children. Of these, 28.9%, 36.7%, and 21.7% had asthma, eczema, and food allergy at 10.5 years, respectively (Table I). Further, 86.8% were sensitized to inhalant allergens at 10.5 years (Table I). Figure E1 (available in this article's Online Repository at www.jaci-inpractice.org) shows the proportion of children who were

consuming farm milk or were exposed to animal sheds at each time point. The consumption of farm milk by children increased from 1 to 3 years and gradually decreased after 4 years. Similarly, exposure to animal sheds also increased from 1 to 4 years and slightly decreased after 5 years.

Temporal pattern of the farm-related exposures on hay fever

Children growing up on a farm had half the risk of hay fever than nonfarm children (aOR 0.50; 95% CI 0.31−0.79; P value .003).

As a first step, we analyzed the temporal pattern of exposure to animal sheds ("continuous exposure to animal sheds," "only early exposure to animal sheds," "only late exposure to animal sheds," and "no exposure to animal sheds"; Figure 1, A) on hay fever development. Of these categories, "only early exposure to animal sheds" showed an inverse association when compared with "no exposure to animal sheds" which however did not reach statistical significance (aOR 0.26; 95% CI 0.06−1.15; P value .08) (Table E2; available in this article's Online Repository at www.jaci-inpractice.org). When adjusting this model for consumption of farm milk exposure classes, the results remained unchanged (Table E2).

We then analyzed the temporal pattern of consumption of farm milk in similar categories "continuous consumption of farm milk," "only early consumption of farm milk," "only late consumption of farm milk," and "no consumption of farm milk" (Figure 1, B). The strongest inverse association was found for the "continuous consumption of farm milk" compared with "no
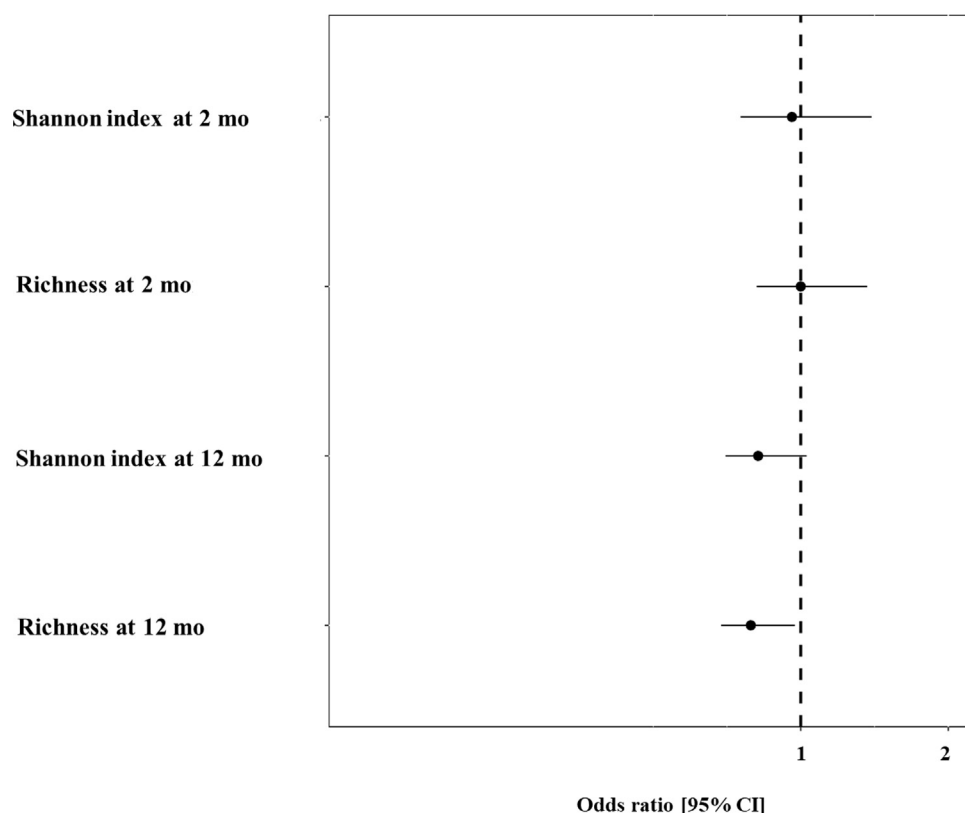
FIGURE 3. Association of gut microbiome richness, and Shannon index at months 2 (hay fever/total: 59/439) and 12 (hay fever/total: 79/633) with hay fever at 10.5 years. Models are adjusted for centers, growing up on a farm, and parental atopy. The association with hay fever is shown as aOR per interquartile range of the probability along with 95% CI.

consumption of farm milk" (aOR 0.35; 95% CI 0.17–0.72; *P* value .004) exposure class (Figure 2 and Table E3; available in this article's Online Repository at www.jaci-inpractice.org). In contrast, "only early consumption of farm milk" showed no significant effect on hay fever. The inverse association of "continuous consumption of farm milk" compared with "no consumption of farm milk" was still observed when using the stringent definition of hay fever (aOR 0.41; 95% CI 0.17–0.97; *P* value .04) (Figure E2; available in this article's Online Repository at www.jaci-inpractice.org) or incident hay fever at 10.5 years (aOR 0.39; 95% CI 0.15–0.99; *P* value .05; data not shown). Because confounding by reverse causation might have biased our findings, we ran a sensitivity analysis in the subgroup of children without a family history of parental asthma and/or atopy and excluded children avoiding milk or milk products at 1 to 6 years. This did not change the inverse association with hay fever (aOR 0.21; 95% CI 0.06–0.78; *P* value .02; data not shown).

We next assessed the association of the frequency of farm milk consumption (ie, whether frequently drinking farm milk has a dose-response effect on hay fever). The highest compared with the lowest quintile of farm milk consumption was inversely associated with hay fever (aOR 0.37; 95% CI 0.16–0.84; *P* value .02), whereas the intermediate group (q2–q4 aOR 0.63; 95% CI 0.37–1.10; *P* value .10) showed a similarly inverse but nonsignificant association. Similar results were obtained when using frequency of farm milk consumption score as a continuous variable (data not shown).

We further investigated whether consumption of processed milk shows similar effects as consumption of farm milk (Figure E3, *A*; available in this article's Online Repository at www.jaci-inpractice.org). Consumption of "high farm and low processed milk" was inversely associated with hay fever (aOR 0.24; 95% CI 0.09–0.66; *P* value .006); however, the consumption of processed milk attenuated the farm milk effect when both farm milk and processed milk were consumed ("mixed consumption of farm and processed milk" [aOR 0.43; 95% CI 0.19–0.96; *P* value .04]) (Figure E3, *B*; available in this article's Online Repository at www.jaci-inpractice.org and Table E3). Furthermore, daily consumption of shop milk at 10.5 years showed association in a positive direction with hay fever (Figure E4; available in this article's Online Repository at www.jaci-inpractice.org).

In addition, NNT calculated in our study was 7.14 (ie, 7 children would have to drink farm milk continuously from pregnancy by mothers until 10.5 years in order to prevent hay fever in 1 child).

Effect of the early-life gut microbiome on hay fever

We investigated the role of the early-life gut microbiome by relating bacterial composition, richness, Shannon index (at 2 and 12 months) and SCFA to hay fever.

We did not find any significant differences in relative abundance of most common bacterial genera at 2 and 12 months with subsequent hay fever at 10.5 year (data not shown). Also, richness and Shannon index of bacteria at 2 months were not
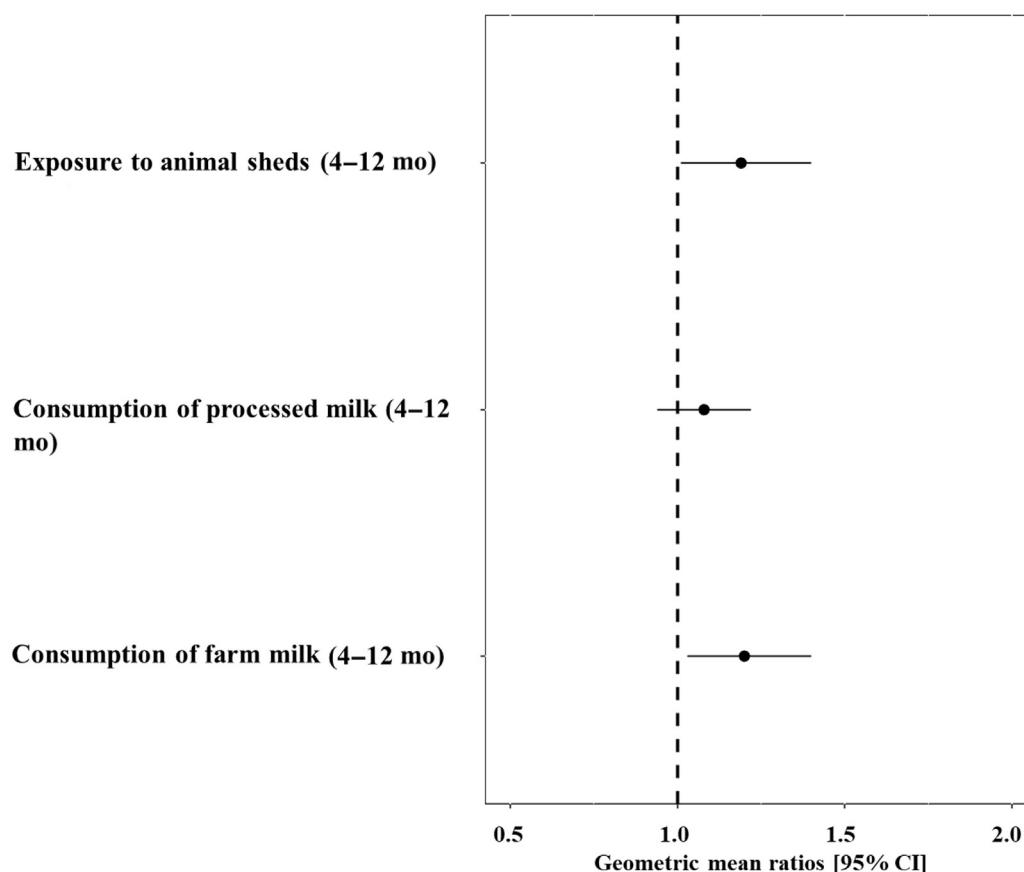
FIGURE 4. Association of consumption of farm milk (n = 624), consumption of processed milk (n = 624), and exposure to animal sheds (n = 617) with richness at 12 months. Models are adjusted for centers, growing up on a farm, and parental atopy. The forest plot represents the aGMRs with 95% CI.

associated with hay fever at 10.5 years (Figure 3). However, the bacterial richness of the gut microbiome at 12 months was inversely associated with hay fever (aOR 0.66; 95% CI 0.46–0.96; *P* value .03; Figure 3). The Shannon index at 12 months also showed an inverse nonsignificant trend for hay fever (aOR 0.71; 95% CI 0.49–1.04; *P* value .08; Figure 3). The SCFAs butyrate (aOR 1.00; 95% CI 0.92–1.09; *P* value .99) and propionate scores (aOR 0.97; 95% CI 0.90–1.05; *P* value .50) were, in turn, not associated with hay fever (data not shown). We reasoned that consumption of milk and exposure to animal sheds may shape the gut microbiome, in particular its richness. Consumption of farm milk (adjusted GMR [aGMR] 1.20; 95% CI 1.03–1.40; *P* value .02) and exposure to animal sheds (aGMR 1.19; 95% CI 1.01–1.40; *P* value .04) in the first year of life increased gut microbiome richness (Figure 4). In turn, no association was observed for consumption of processed milk (Figure 4). Because both farm milk consumption and exposure to animal sheds during infancy (4–12 months) showed significant associations with gut microbiome richness at 12 months, we performed a mediation analysis including unexposed and children exposed to both in infancy. The mediation analysis revealed that part (18.4%) of the total protective effect of farm milk consumption and exposure to animal sheds in the first year of life on hay fever was mediated by gut microbiome richness (*P* value .03; Figure 5). The number of children only being

exposed to animal sheds or farm milk, respectively, was too low to allow separate mediation analyses.

DISCUSSION

In the PASTURE birth cohort, the continuous consumption of farm milk up to 10.5 years, but neither the only early nor the only late exposure alone, was significantly associated with reduced risk of hay fever at 10.5 years. In contrast, exposure to animal sheds only exerted a trend toward protection early in life. Both exposures, farm milk and animal sheds, early in life increased gut microbiome richness at 12 months, which partly explained the protective effect of these exposures on hay fever.

The human gut microbiome composition plays an important role in shaping the development of the immune system.[24] There is some evidence that the gut microbiome diversity in the first years of life may protect from atopic sensitization. In the population-based CHILD cohort, the Shannon index at 3 months was associated with protection from atopic sensitization at 1 year.[8] However, in a Swedish study, the Shannon index in early infancy was not associated with allergic rhinoconjunctivitis and SPT at 7 years.[25] Our analyses likewise do not confirm this very early window of opportunity because gut microbiome richness and Shannon index at 2 months was unrelated to hay fever development.

Total effect, β [95% CI] = -0.98 [-1.88 to -0.08]; P value = .03

Indirect effect, β_2 [95% CI] = -0.18 [-0.36 to -0.004]; P value = .03

Direct effect, β_1 [95% CI] = -0.80 [-1.70 to 0.10]; P value = .08

FIGURE 5. Mediation analysis of the protective effect of consumption of farm milk and exposure to animal sheds in infancy on hay fever mediated by gut microbiome richness at 12 months adjusting for centers (n = 466). The figure shows the direct (β_1), indirect (β_2), and total (β) effects as well as their respective 95% CI from the path model. The proportion of the mediated (indirect) effect was 18.4%.
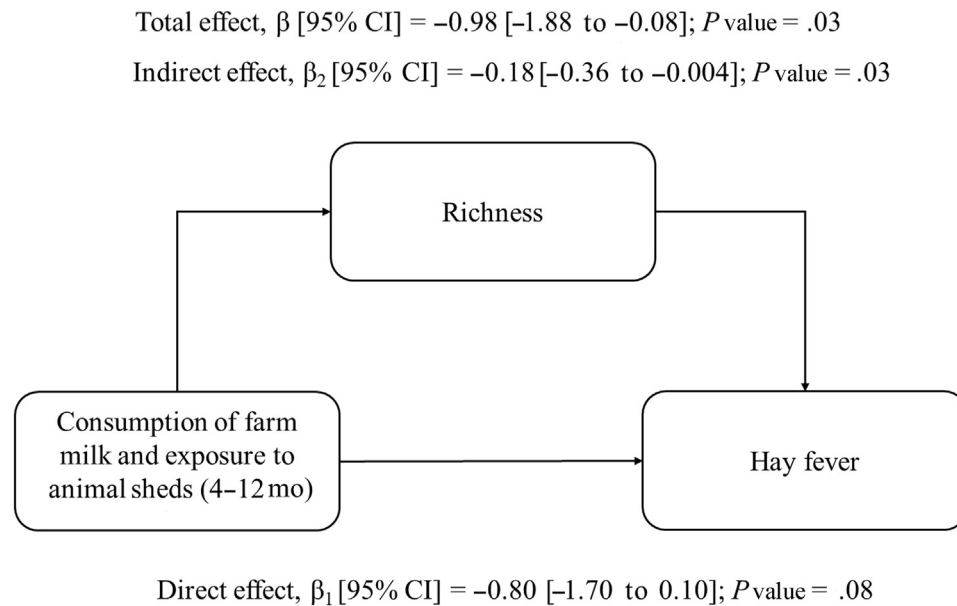
In contrast, gut microbiome richness at 1 year was inversely associated with hay fever at 10.5 years. We have previously shown in the PASTURE cohort in agreement with others that the compositional structure of the gut microbiome undergoes very significant changes from early age, when most infants are breastfed, to 12 months, when most foods have been introduced into a child's diet.[10,11] Nevertheless, an inverse association of gut microbiome richness at 1 year with an outcome much later in life at 10.5 years may seem surprising. This long-term association may be attributable to an earlier onset of disease. In fact, 4.6%, 5.9%, and 6.7% of children with data on hay fever at 10.5 years had already reported symptoms and/or a diagnosis of hay fever at 4, 5, and 6 years, respectively. Furthermore, early alterations of the composition of the gut microbiome may shape its subsequent development toward an adult-like compositional structure in the first 3 years of life.[26] Unfortunately, no fecal samples have been collected at later time points in the PASTURE cohort.

The production of the SCFAs butyrate and propionate measured at 12 months has been reported previously as determinants of protection against atopic sensitization at 6 years.[20] In our study, no relation between the SCFAs butyrate and propionate with hay fever was found. Furthermore, no association with single taxa was seen. Thus, different facets of the early development of the gut microbiome composition may matter for different clinical outcomes.

Of the environmental exposures investigated in these analyses, the continuous, but neither the early nor the late, consumption of farm milk was seen to protect from hay fever development. Moreover, a dose-response effect was found corroborating the strength of the observation. Interestingly, this protective effect was partly mediated by gut microbiome richness, which may suggest that a continued exposure to unprocessed cow's milk may increase gut microbiome richness beyond 12 months and thereby confer its protective effect.

Continuous exposure also implies repeated exposures. The novel concept of trained immunity may lend itself to mechanistic speculations because phenomena like lipopolysaccharides tolerance are based on the necessity of repeated rather than single exposures.[27]

A potential explanation for the differential effect of unprocessed versus processed cow's milk is grounded in the observation that most farm children drink their milk unboiled. In fact, too few children received only boiled (ie, heat-treated farm milk) over the study period to allow meaningful stratified analyses. A number of population-based and experimental studies have stressed the potential importance of heat treatment of cow's milk for the loss of protective effects.[16,28-31] Whether alterations of the milk microbiome or denaturation and loss of function of milk (whey) proteins underlie these findings awaits further elucidation.

Exposure to animal sheds during early years showed an inverse, albeit nonsignificant effect on hay fever. This is in contrast to previous farm studies showing stronger effects.[12,32] The discrepancy might be attributable to important differences in the definition of exposure to animal sheds used in the PASTURE study, which only assessed exposure to any animal sheds without differentiating between cows, pigs, sheep, and horses. The nature of animal exposure may matter, however. Whereas exposure to cow sheds showed a significant protective effect on hay fever and asthma,[12] sheep sheds and keeping of hares and rabbits were risk factors for wheezing and asthma, respectively, in the PARSIFAL farm study.[33]

The main strength of this study is its longitudinal design, which enabled us to assess the exposures at several time points

before the assessment of the outcome. Excluding children with parental asthma and/or atopy and who were avoiding milk or milk products showed similar inverse associations with hay fever, consequently arguing against confounding by reverse causation. An elevated risk of diarrhea and farm milk consumption at 10.5 years was not observed (data not shown). The results of the present study show protective association of continuous consumption farm milk on hay fever. However, one of the potential caveats of the observation study is finding causality. Hence, the Milk Against Respiratory Tract Infections and Asthma (MARTHA), an ongoing interventional trial, is being carried out to evaluate the preventive effect of minimally treated (ie, only pasteurized and, thus, microbiologically safe) cow's milk on upper respiratory tract infections and allergy.[34] Further, the NNT in our study was 7; however, this study is not a randomized placebo-controlled double-blind trial, and thus, numbers must be taken with some caution. One of the drawbacks of the study is the missing data on hay fever at 10.5 years. However, comparing the baseline characteristics between included and excluded children did not show any significant difference except for maternal age at pregnancy, maternal smoking, parental education, and premature birth. However, adjusting for these variables did not change the results (data not shown). Another drawback is the small number in the "only early" and "only late" exposure groups that shows protective nonstatistical significant effect on hay fever. However, using the RMLCA approach, our study could identify these small groups manifesting that these types of habits (ie, farm milk consumption or exposure to animal sheds) do exist. We performed a *post hoc* power calculation using SAS and considering α of 0.05 (2-sided). For our sample size of 650 (ie, in the exposure groups "continuous consumption of farm milk" and "no consumption of farm milk"), the power of study is over 80% assuming the response probabilities ranging from 0.02 to 0.18 for having hay fever in children who consume farm milk and unadjusted odds ratio of 0.24. Thus, our study was well powered to detect a relatively strong effect of farm milk consumption on hay fever.

In summary, the results of the present study demonstrate that continuous exposure of the main determinant (ie, farm milk consumption) but neither only early nor only late exposure alone conferred protection from hay fever development. The early compositional structure of the gut microbiome at 1 year, but not 2 months, did, however, in part mediate this protective effect. One might speculate that continuous consumption of unprocessed cow's milk may also increase gut microbiome richness at later ages, but we do not have data to support this notion. Overall, the findings presented herein do not support the notion of an early-determined trajectory in which only early exposures in the first months of life would govern later outcomes. These results emphasize the preventive potential of continuously drinking unprocessed farm milk for hay fever protection. However, the risks associated with raw cow's milk consumption prohibit its recommendation for daily life. The results of the MARTHA trial, however, will shed light on potential side effects.[34] Further clinical trials based on the present results are warranted.

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