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Prevention of allergies and infections by minimally processed milk in infants—The MARTHA feasibility and safety trial

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Abstract

Background: Consumption of raw cow's milk has repeatedly been shown to protect from asthma, allergies, and respiratory infections. As raw milk bears potential health hazards, it cannot be recommended for prevention. Therefore, we performed an intervention study with microbially safe but otherwise minimally processed cow's milk. Here we describe feasibility and safety of the trial.

Methods: The MARTHA trial (DRKS00014781) was set up as a double-blind randomized intervention in a population residing in Bavaria. Infants from 6 to 36 months of age consumed minimally processed cow's milk (intervention arm) or ultra-heat-treated (UHT) semi-skimmed milk (comparator arm).

Results: At the age of 6 to 12 months, 260 infants were enrolled, with 72% having a family history of atopy. The extensive screening system for milk consumption and symptoms suggestive of adverse events was well accepted with 22,988 completed weekly surveys and an average completion of 82% surveys sent out. The children consumed the study milk on average on 457 days (61% of intervention days). The intervention proved to be safe without any case of milk allergy or milk intolerance under the intervention in both arms. All 6 cases of serious adverse events were unrelated to milk. The most common reason was unscheduled hospitalization of more than 3 days.

Conclusions: The intervention with minimally processed milk and the study instruments proved feasible. During the age of 6 to 36 months, there was no increased risk of milk allergy in a population with a substantial proportion of family history of atopy.

KEYWORDS
asthma, asthma: infectious diseases, food allergy, food allergy: cow's milk, nutrition

1 | INTRODUCTION

Children living on traditional farms suffer less from asthma, allergies, and viral infections of the airways as shown by numerous studies across the globe.[1] This effect has partially been attributed to the consumption of unprocessed cow's milk instead of industrially processed, high-heat treated milk.[1–3] Various milk ingredients have been suggested to mediate the effect including polyunsaturated fatty acids, whey proteins, milk fat globule membranes, oligosaccharides, microRNA, immunoglobulins, and microbial components.[1,4–6] Population-based studies, however, failed to attribute the beneficial effect of cow's milk to specific molecules, because milk is a heterogeneous mixture of thousands of components.[1] Moreover, cow's milk is subjected to various industrial processing steps and modes of storage before consumption.[1] These conditions render an explanation of the beneficial effect on a molecular basis almost impossible. Although there are several observational studies on the beneficial effects of raw farm milk, its use in young infants cannot be recommended because raw milk bears the risk of transmitting life-threatening infections. Therefore, no interventions with raw milk have been performed so far.

We hypothesized that the beneficial ingredients of native milk are (at least partially) preserved in minimally processed but microbially safe milk. The aim of the Milk Against Respiratory Tract Infections and Asthma (MARTHA) trial was to verify the previously described observations on protection from asthma, allergies, and infections in a randomized placebo-controlled double-blind trial with atopic sensitization at 3 years as primary endpoint.

Due to the SARS-CoV-2 pandemic, we experienced difficulties with recruitment under lockdown conditions and a shift of public funding towards COVID-19 as the prevailing respiratory disease. In 2021, the Data Safety and Monitoring Board (DSMB) recommended stopping recruitment and continuing the trial as a feasibility study during the remaining funding period. At this point, 260 out of the originally planned 960 children were enrolled.

Though the administration of cow's milk was according to the national recommendations for child nutrition, questions on tolerability of cow's milk in infants and its potential risk of triggering milk allergy remain. The aim of the present analysis was to determine the participants' adherence to the intervention and the tolerability of cow's milk early in life. For a safety analysis, data of 260 children with an average intervention duration of about 2 years were analyzed. Results on primary and secondary outcomes will be reported separately.

Key message

Intervention with minimally processed cow's milk in children 6 to 36 months old was feasible with a high retention rate and no increased risk of milk allergy.

2 | METHODS

2.1 | Study design and population

The MARTHA trial was set up as a double-blind randomized placebo-controlled intervention with minimally processed cow's milk versus semi-skimmed UHT milk (Figure S1). The primary endpoint was atopic sensitization to food or inhalant allergens at age 3 years. Inclusion criteria were healthy infants of both sexes between 6 and 12 months and written informed consent provided by parents or guardians. Major exclusion criteria were gestational age below 35 weeks and preexisting adverse conditions such as sensitization to cow's milk, bronchodysplasia, cystic fibrosis, immune deficiency, or failure to thrive. During a pilot phase, the clinical center in Regensburg included 12 participants but was closed because of the SARS-CoV-2 pandemic; its participants were taken over by the sole remaining clinical center in Munich.

The trial was approved by the ethics committee at the Medical Faculty of LMU Munich (18-0405) and registered under the trial number DRKS00014781 (universal trial number U1111–1216-7580) with the German clinical trials registry (DRKS, https://drks.de).

2.2 | Recruitment and clinical visits

Trained field workers approached families in birth clinics, and written informed consent was obtained for processing individual contact data. Families were invited for Clinical Visit (CV) 1 at the child's age of 6 to 12 months, and written informed consent to the participation in the entire trial was obtained at CV1. The infants were checked for exclusion criteria such as failure to thrive, gestational age below 35 weeks, cow's milk intolerance, and cow's milk allergy. The latter was suspected if there were typical symptoms as listed in Table 1 and an allergen-specific immunoglobulin E (sIgE) to any cow's milk allergen ≥0.7 kU/L or, irrespectively of symptoms, ≥3.5 kU/L was observed. The children were electronically randomized to either the intervention or comparator arm. Randomization was performed by CASTOR (see below) and based on a permuted balanced block design with random block length considering stratification by study site. CV1 took place from August 2019 until October 2021.

CV2 took place independently of age during the winter season 2021/2022 to assess respiratory infections after the lockdown periods.

At the end of the intervention, CV3 was performed. CV2 and CV3 used identical instruments with respect to questionnaires and biosampling (venous blood, nasal swabs, and fecal samples). The last CV3 was completed on 2023-06-30.

2.3 | Intervention

The intervention consisted of daily consumption of minimally processed full-cream milk, which was pasteurized for 20 seconds at

TABLE 1 Symptoms suggestive of cow's milk allergy, cow's milk intolerance, and lactose intolerance.

Onset	Symptoms
Immediate (within 2 h after milk consumption)	• Skin: rash (urticaria, eczema, erythema) • Respiratory: cough, wheezing, breathing difficulties • Gastrointestinal: vomiting or diarrhea • Edema: swelling of the face, especially the lips
Delayed (within 4 h after milk consumption)	• Worsening of an existing eczema
Lasting symptoms (several days)	• Constipation with daily problems for more than 2 weeks • Diarrhea without fever on at least 5 consecutive days • Bloody stool, vomiting on at least 4 consecutive days

72 °C temperature (according to legal requirements), but was not otherwise treated, that is, not separated and not homogenized. The comparator group received the same amount of UHT semi-skimmed milk, which was most commonly given to infants in observational studies in Germany.[1] Semi-skimmed milk is also recommended in Germany.[7,8] Considering the difference in energy content, additional feeding was ad libitum.

According to the German nutritional recommendations,[7–10] children received 200 ml milk from 6 months onwards. In children still exclusively breastfed the start of the intervention was delayed until supplemental feeding was initiated by the parents. At the age of 10 months, when a second milk meal is introduced according to the recommendations, the daily dose was changed to 2 × 150 ml. To comply with the nutritional recommendations, we discouraged the consumption of additional milk, particularly raw milk, and products thereof.

During a pilot phase from August 2019 until September 2020, both arms received cow's milk prepared from spray-dried milk (FrieslandCampina, The Netherlands). Thereafter, children were fed liquid milk from regional providers. Both, the intervention and comparator products were packed in neutral containers with only the study logo and a letter code printed on and shipped under persistent cooling by a distributor of regional farm products. Blinding of the milk products was verified by professional tasters and by the study team (failure to identify the products by tasting).

2.4 | Questionnaires

The parents were asked to complete short electronic questionnaires on a weekly basis. These questionnaires contained questions on consumption of study milk, other milk types, and other major foods of infant diet. The questionnaires also collected data on symptoms suggestive of milk intolerance or allergy and symptoms of respiratory tract infections such as rhinitis, cough, and wheezing, as well as fever (temperature ≥38.5°C) or elevated temperature (≥37.5°C). For the safety outcomes, the surveys were continued for 4 weeks after completion of the intervention. Every 13 weeks of life, the weekly surveys were complemented by additional questions, which we called "quarterly surveys." These contained items on height and weight, nutrition, environmental exposures such as other children or pets, and potentially serious adverse events like hospitalization and positive COVID-19 PCR tests. The feasibility of the weekly questionnaire was assessed in a pilot phase using a different system in 56 individuals with altogether 336 surveys in the first 5 months, during which no adverse events were observed. The current analysis includes only data thereafter, that is, from 01 January 2020 onwards. An optional survey on additional population characteristics such as family attitudes towards nutrition, particularly milk consumption, characteristics of housing, smoking habits, parental education, and birthplace of parents, was issued on 07 December 2021 to the 210 participants enrolled at that time.

2.5 | Study endpoints

The primary endpoint was atopic sensitization at age 3 years, defined as sIgE ≥0.7 kU/L to food or inhalant allergens at CV3. For this purpose, a standard allergen panel was measured at the Department of Clinical Chemistry of LMU Hospitals Munich. Missing biosamples at CV3 were replaced by CV2 biosamples if CV2 occurred no more than 10 months from the third birthday. This was possible because CV2 and CV3 used the same study instruments and differed only by time point of performance.

Secondary endpoints were sensitization at CV2, high-sensitivity CRP at CV3, atopic eczema until age 3 years, wheeze and infections during intervention, gut microbial composition, and maturation during the intervention.

2.6 | Safety outcomes

Adverse events (AE) were defined as cow's milk allergy, cow's milk protein intolerance, and lactose intolerance. Study physicians evaluated suspicious symptoms according to a predefined algorithm (Figure 1). Queries were triggered by the electronic data capture form (eCDF) system, when the parents entered one or more of the symptoms listed in Table 1.

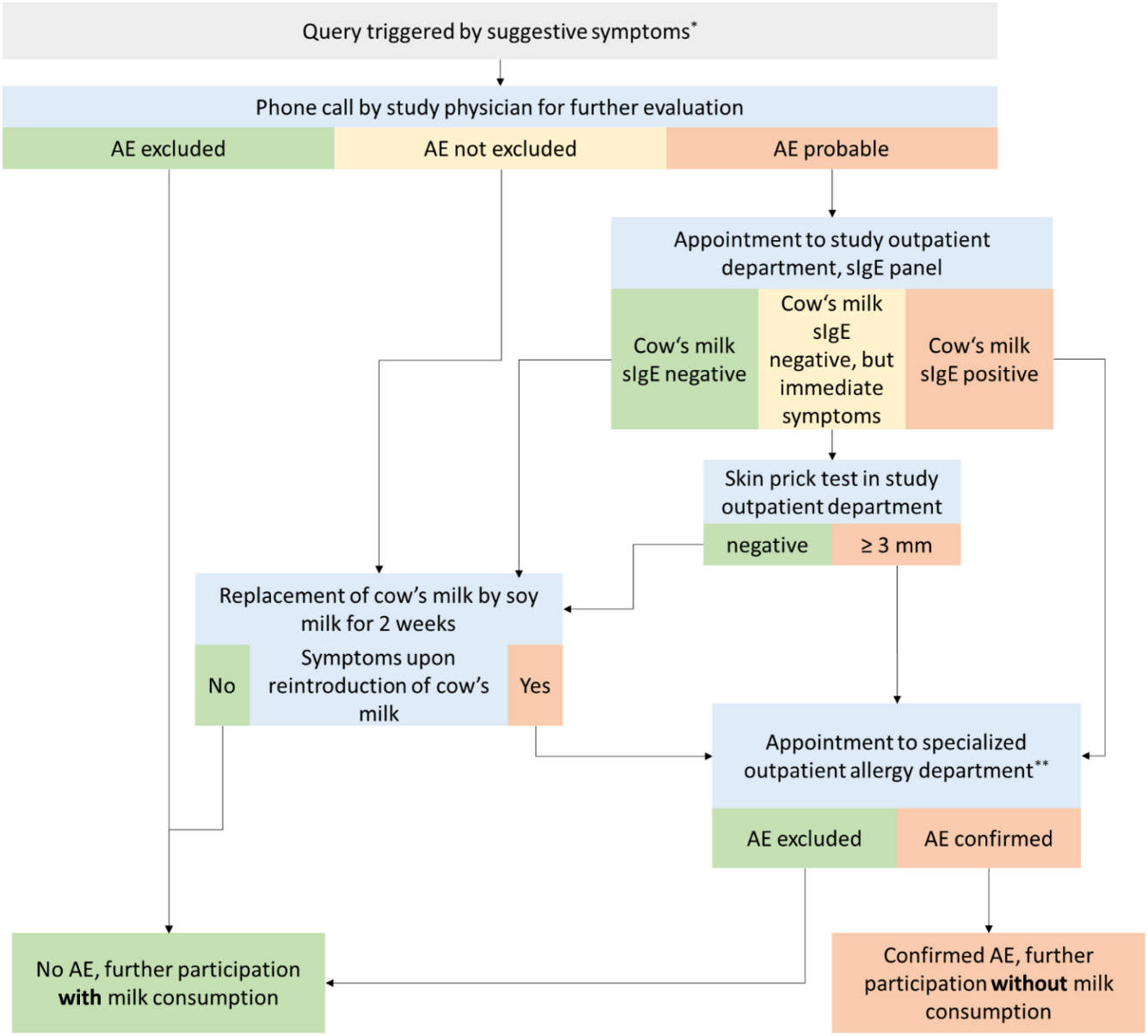

FIGURE 1 Workup flow of potential AEs in relation to cow's milk. AE, Adverse event; sIgE, allergen-specific Immunoglobulin E. *Query created by system once one of the symptoms listed in Table 1 was ticked in the survey. **Further clinical evaluation of potential AEs including food challenge tests where necessary.

Upon electronic notification of queries, the study physicians called the families for detailed information. If the physicians found a trivial explanation such as an erroneous diary entry or a self-limiting gastrointestinal infection affecting also other family members, an AE was considered excluded. If they judged the symptoms as probable AE, an additional study visit was scheduled for a clinical workup with blood taking. A positive sIgE (defined as ≥0.7 kU/L) for milk allergens (F2), α-lactalbumin (F76), β-lactoglobulin (F77) or casein (F78) was considered an indication for an oral food challenge (OFC) performed under clinical conditions in the specialized allergology department of Dr. von Hauner Children's Hospital according to their inhouse standard operating procedures. In case of a negative sIgE but a clear temporal relationship between milk ingestion and symptoms or if blood sampling failed, a skin prick test (SPT) was performed for evaluation. A wheal size for cow's milk of 3 mm or more was an indication of OFC.

In all other children, including children where an AE could not be excluded during the initial call, cow's milk was replaced by soy milk for 2 weeks, and symptoms were assessed upon reintroduction of cow's milk. Reoccurrence of symptoms was an indication for OFC. Otherwise, consumption of cow's milk was continued and cow's milk allergy and intolerance were excluded.

Serious adverse events (SAE) were defined as unscheduled hospitalization for more than 3 days; hospitalization for a SARS-CoV-2 infection of any duration; and failure to thrive defined by weight percentiles below 3% or by a decline of more than 20 percentiles within 3 months. If a survey was not completed or information on weight was missing, a study nurse contacted the family to collect the missing information.

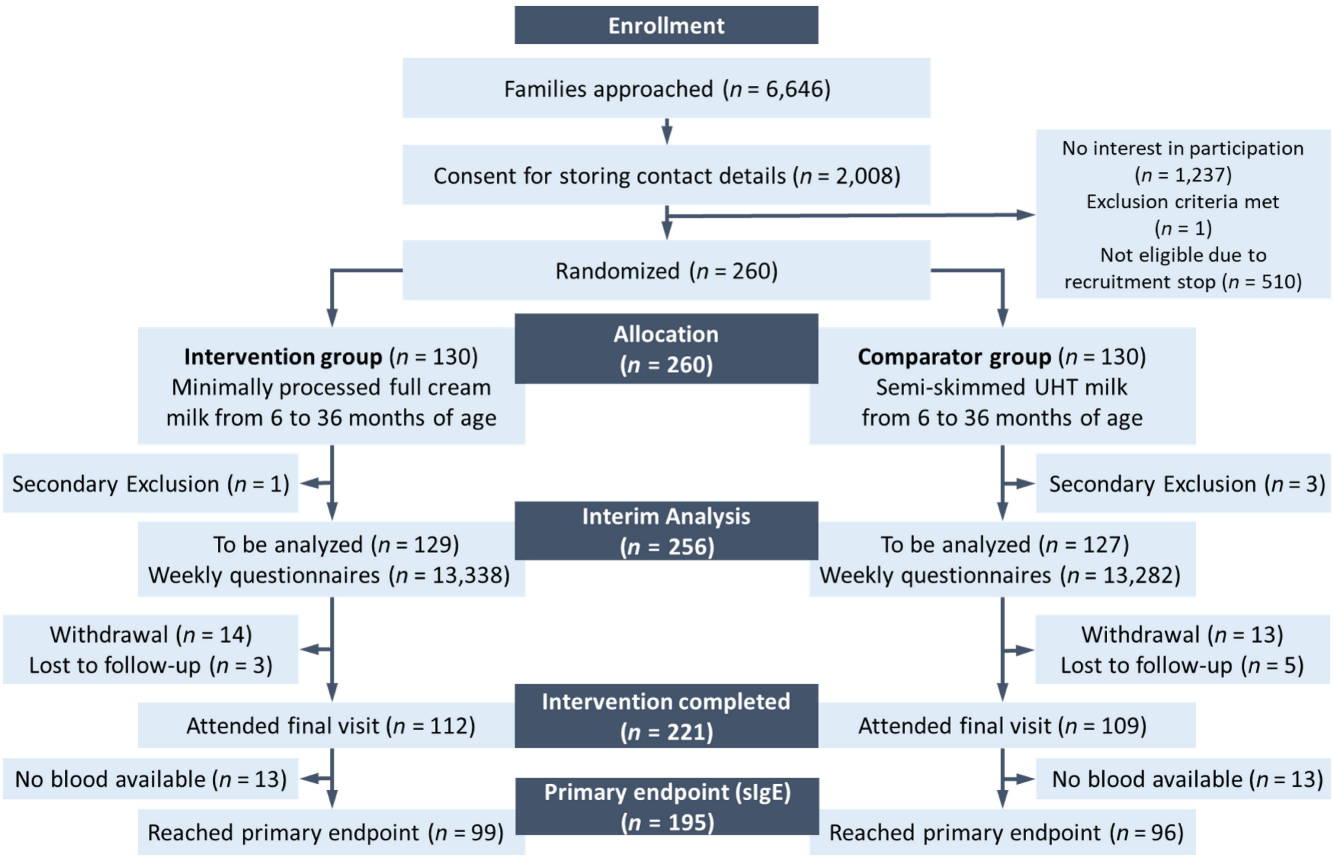

FIGURE 2 Participant flow. Flow chart according to CONSORT.[24] Exclusion for suspected milk allergy based on positive milk sIgE: $n=1$ before randomization (primary), $n=1$ in intervention and $n=2$ in comparator arm. One child in the comparator arm was excluded for gestational age below 35 weeks.

Study physicians checked whether hospitalizations were due to a SARS-CoV-2 infection or longer than 3 days and whether there could be a relation to milk consumption. If physicians were notified by the system about low or lowering weight percentiles, they evaluated the weight development also by checking weight measurements verified by a health professional during standard check-ups and, if necessary, contacted the family pediatrician with parental consent for concurrent diagnoses. All SAEs were discussed with the principal investigator, who evaluated the SAEs for a potential relationship with the study milk and decided whether milk consumption was continued.

2.7 | Data management

All clinical data were collected by the electronic data capture system CASTOR EDC (Ciwit B.V., Amsterdam, The Netherlands) and stored under a record identifier. Personal details such as names and addresses were stored in encrypted fields, which could be decrypted only by the clinical team and the staff involved in mailing the study milk.

Clinical questionnaires and reports were completed by trained clinical staff. Weekly surveys were answered by parents directly entering the data through a protected channel into the CASTOR system. The answers were screened for symptoms suggestive of S/AEs by R scripts communicating at least twice a week with CASTOR through its application programming interface. Automatically triggered E-mail notifications informed the study physicians immediately. The entire process was monitored weekly by a clinical research associate.

Clinical data were extracted on 11 January 2023, and a data trustee outside LMU Hospitals replaced the record identifier with a second pseudonym for subsequent statistical analysis.

2.8 | Statistical analysis

Characteristics of the study population, adherence to the protocol, and occurrence of S/AE were assessed descriptively and, if relevant, compared between the study arms by Fisher's exact test or Student's *t*-test. The distribution of potential confounders was compared between study arms. Statistical analyses were performed with R version 4.1.2.[11]

3 | RESULTS

We approached 6,646 families, of whom 30% consented to storing their contact details and 17% were still interested in the trial at recruitment age (Figure 2). Of the 260 randomized participants, 85%

TABLE 2 Distribution of potential confounders.

Characteristics	Complete data ($n=260$)	Both arms ($n=260$)	Intervention arm ($n=130$)	Comparator arm ($n=130$)	p-value
Female gender	260	126 (48%)	**55 (42%)**	**71 (55%)**	**.049**
Maternal atopy	255	111 (44%)	55 (43%)	56 (44%)	.772
Paternal atopy	247	132 (53%)	61 (50%)	71 (56%)	.352
Parental atopy	250	181 (72%)	87 (69%)	94 (76%)	.234
Older siblings	260	108 (42%)	57 (44%)	51 (39%)	.452
Contact to pets in family	243	95 (39%)	47 (38%)	48 (41%)	.624
Cow's milk in pregnancy	260	248 (95%)	122 (94%)	126 (97%)	.239
Gestational age in months	260	39 (34–42)	39 (35–42)	39 (34–42)	.102
Gestational diabetes	260	19 (7%)	10 (8%)	9 (7%)	.812
Maternal age in years	260	34 (20–47)	34 (20–46)	34 (21–47)	.607
Cesarean section	260	99 (38%)	48 (37%)	51 (39%)	.702
Premature contractions	260	14 (5%)	6 (5%)	8 (6%)	.584
Birth weight in grams	260	3.370 (1.770–4.535)	3.350 (2.130–4.400)	3.400 (1.770–4,535)	.217
Antibiotics ever	260	15 (6%)	8 (6%)	7 (5%)	.791
Atopic eczema before intervention	247	11 (4%)	5 (4%)	6 (5%)	.727
Cow's milk before intervention	260	129 (50%)	66 (51%)	63 (48%)	.711
Infant formula before intervention	260	175 (67%)	89 (68%)	86 (66%)	.693
Age at start of intervention in weeks	257[a]	35 (26–64)	34 (26–64)	35 (26–61)	.318
Age at end of intervention in weeks	257[a]	149 (32–156)	150 (32–156)	147 (36–156)	.847
Weight percentiles at CV1	260	53.3 (27.59)	55 (26.66)	51.7 (28.5)	.386
Weight percentiles at CV2	215	56.1 (28.63)	55.8 (28.89)	56.4 (28.51)	.893
Weight percentiles at CV3	193	52.6 (27.9)	51.2 (28.71)	54.2 (27.06)	.46
Height percentiles at CV1	260	41.5 (28.85)	42 (27.65)	40.9 (30.09)	.584
Height percentiles at CV2	216	44.3 (30.56)	44.3 (30.87)	44.3 (30.4)	.929
Height percentiles at CV3	193	51.1 (30.3)	50.4 (31.41)	52 (29.17)	.635

Note: Significant differences are printed in bold. Values missing in no more than 5% of included children. Frequencies are given with percentages in brackets and for continuous variable median and range except for percentiles where mean and standard deviation are given.

[a]Three children did not start the intervention due to withdrawal of consent, secondary exclusion for increased sIgE and secondary exclusion for prematurity.

completed the intervention with a final visit and 75% reached the primary endpoint sIgE after the intervention. Four children were excluded for suspected cow's milk allergy or intolerance (Table S1), whereas three non-symptomatic children had slightly elevated sIgE to cow's milk (<3.5 kU/L), thus not meeting the exclusion criteria (Table S2).

Family history of atopic conditions was rather common affecting 72% of the study population (Table 2). Major potential confounders were equally distributed between the study arms by randomization except for sex.

The additional questionnaire on population characteristics ($n=204$ completed surveys of the 210 invited families) reported a diverse population with 31% of individuals having a parent born outside Germany and a high socioeconomic status as suggested by 98% of mothers with at least 10 years of education (Table S3). The overall satisfaction with the organization of the study and the communication with study staff was rated very good or good in over 90% of the participating families (Table S4).

On average, children completed 107 weeks of intervention (2 years) and had 82% of diaries completed (Table 3). The intervention milk was consumed on at least 365 days by 73%. The amount of intervention milk consumed varied with age but not significantly between study arms though a slight tendency was noted (Figure S2).

Symptoms suggestive of AEs were reported in 2% of diaries (Table 4) with diarrhea (0.87%) and constipation (0.48%) being most common (Table S5). The 546 queries triggered by the suspect diary entries for an AE were worked up with iterative phone calls to 182 participating families. In 4 cases, an additional study visit was

TABLE 3 Adherence.

Criterion	Both arms ($n=260$)	Intervention arm ($n=130$)	Comparator arm ($n=130$)
Overall duration of intervention (weeks)[a]	107 (91–127)	109 (91–124)	105 (90–130)
Duration of intervention until CV2 (weeks)[a]	67 (44–96)	66 (45–95)	68 (43–97)
Completion of weekly surveys			
90% of weekly surveys	143 (55%)	73 (56%)	70 (54%)
50% of weekly surveys	235 (90%)	119 (92%)	116 (89%)
Average proportion of weeks	82%	83%	81%
Consumption of intervention milk			
Average of days (any amount)	457	451	462
Any amount on at least 365 days	189 (73%)	96 (74%)	93 (72%)
Entire serving on at least 365 days	76 (29%)	35 (27%)	41 (32%)

Note: Intention to treat population, that is, all randomized children comprising those with secondary exclusion ($n=1$ in the intervention arm and $n=3$ in the comparator arm).

[a]Median and first quartile to third quartile.

performed for sIgE measurement or SPT (Table 4). In 42 children, cow's milk was replaced by soy milk, and all but one lost their symptoms (Table 4). The child with continued symptoms was referred to the specialized outpatient department for a food provocation test but proved negative.

Of the three children included despite mild sensitization to milk (Table S2), none developed symptoms suggestive of cow's milk allergy throughout the intervention. One of them, however, had increasing milk sensitization (F2) up to class 4 (≥17.5 kU/L) at CV3, but the absence of typical symptoms was confirmed by study physicians. An additional participant also showed elevated sIgE against cow's milk (F2) of class 4 only at CV3 but was equally free of symptoms as verified by study physicians. A third one had class 3 sensitization (≥3.5 kU/L) to α-lactalbumin (F76) only at CV3. Our study physicians affirmed no symptoms for a cow's milk allergy after speaking to the parents, however, they advised to clarify a suspected nut allergy with respective sIgE values being in class 3.

Flags for potential SAEs were raised in 22% of the quarterly questionnaires (Table 4). SAEs were confirmed in 1 child of the intervention and 5 children of the comparator arm (Table 4). According to a consensus meeting of all involved clinicians, all six cases of SAE were unrelated to milk consumption.

4 | DISCUSSION

The MARTHA feasibility trial showed that cow's milk was tolerated by infants from 6 months onwards. None of our study participants experienced an AE defined as cow's milk allergy, cow's milk intolerance, and lactose intolerance. The drop-out rate was very moderate, and the weekly diaries were well accepted by the participating families.

The weekly questionnaires were inspired by those of the PASTURE study, which proved useful for assessing infections and wheezing in the first year of life.[3,12,13] For the MARTHA trial, we implemented additional questions with a focus on typical symptoms of milk allergy and intolerance. The automated communication with the database allowed screening for suggestive symptoms in more than 200 participants simultaneously. By this instrument, we could not only monitor potential AEs and adherence to the diaries but also control (self-reported) milk consumption. So, we could keep track of mailing cooled milk and replace lost milk packages immediately. A major advantage of the weekly surveys and the resulting relatively frequent phone calls ($n=7{,}729$ in total) was the intensive contact with the participating families, which contributed to the low attrition and allowed for comparably few clinical visits.

The intervention itself was feasible with respect to the regularity of consumption of the intervention milks. The daily dose, however, was hardly consumed by all participants. The higher fat content in the intervention milk led to slightly lower milk consumption in this group. This may suggest that the daily dose could be ad libitum in subsequent studies.

Randomization is actually used to distribute known and unknown confounders equally between the study arms, which is more successful with large sample sizes. In smaller sample sizes, stratified randomization can be used to prevent imbalance in potential confounders.[14] Because of technical issues and a much higher anticipated sample size, randomization was only stratified for center in the current study. This resulted in an unbalanced distribution of sex, a known determinant of childhood allergies. Potential confounding by sex should be considered in future statistical analyses of the primary and secondary endpoints. Here we performed a descriptive analysis of the safety outcomes, which does not require an adjustment.

TABLE 4 Safety outcomes.

Criterion	Both arms ($n=260$)	Intervention arm ($n=130$)	Comparator arm ($n=130$)
Adverse events			
Weekly surveys completed	22.988	11.662	11.326
Weekly surveys flagged	546 (2.38%)	259 (2.22%)	287 (2.53%)
Additional study visit	4	4	0
Milk-specific IgE measured	2	2	0
Skin prick test	3	3	0
Discontinuation of cow's milk	42	17	25
Loss of symptoms under soy milk	41	16	25
Symptoms upon reintroduction of cow's milk	0	0[a]	0
Food challenge required	1	1	0
Milk-specific IgE (F2) ≥class 3 at CV3	2	1	1
Confirmed adverse event	0	0	0
Serious adverse events			
Quarterly surveys completed	1.691	841	850
Flag triggered	378 (22%)	196 (23%)	182 (21%)
Hospitalization more than 3 days	45	21	24
Hospitalization for COVID-19	127	59	68
Failure to thrive	206	116	90
Confirmed serious adverse event	6	1	5
Hospitalization more than 3 days	3	0	3
Hospitalization for COVID-19	1	0	1
Failure to thrive	2	1[c]	1[b]
Relation to milk consumption	0	0	0
Number of children affected (%)	6 (2%)	1 (1%)	5 (4%)

Note: Intention to treat population, that is, all randomized children comprising those with secondary exclusion ($n=1$ in the intervention arm and $n=3$ in the comparator arm).

[a]One child continued with symptoms under soy milk and could not be evaluated for reintroduction of cow's milk.

[b]Possibly celiac disease.

[c]Attending pediatrician assumed normal development below the third percentile without aggravation under continued cow's milk consumption.

Although we excluded children with positive sIgE against cow's milk at CV1, there might have been a certain risk of cow's milk allergy emerging under the intervention. A systematic review of European studies from 2000 until 2012 reports a prevalence of 6.76% [5.96%–7.55%] in children aged 2–5 years for sIgE-positive cow's milk allergy.[15] Based on these figures we would have expected in our sample of 260 children 15 to 20 cases or probably more because of the common family history of atopy in our study population. Despite scrutinized surveillance with weekly diaries and a thorough work-up by study physicians, we did not detect any case of milk allergy beyond the 4 children who were excluded at CV1. Despite the limited sample size, these figures render the induction of cow's milk allergy by a milk intervention very unlikely. For high sensitivity, we chose a cut-off of 0.7 kU/l, which is relatively low compared to the recommendations.[16]

To cover various types of reactions to cow's milk, we screened for symptoms at different time intervals after milk ingestion. First, we considered immediate IgE-mediated reactions by focusing on the first 2 h or, in case of deterioration of skin symptoms, 4 h after ingestion. Second, we covered delayed non-IgE-mediated reactions up

to 48 hours as in cow's milk protein intolerance. In very rare cases, bloody stools may indicate cow's milk intolerance early in life—even if exposure to cow's milk occurs through breastfeeding.[17,18] Therefore, we also screened for bloody stools as an indicator of cow's milk intolerance. Possible manifestations may affect the skin (e.g., urticaria, eczema, erythema, oral, or perioral rash), the respiratory tract (e.g., cough and/or wheezing and extremely rarely laryngeal edema), and the gastrointestinal tract (e.g., oral or perioral swelling, diarrhea, vomiting, constipation, refusal to eat, and failure to thrive). Also, acute systemic reactions may appear such as anaphylaxis.[19]

Symptoms of lactose intolerance may be diarrhea, abdominal pain, flatulence, nausea, and/or bloating.[20] In addition, the age of lactose intolerance manifestation differs among ethnicities.[20] Although down-regulation of the physiological expression of lactase can already be seen from the second year of life,[21] the disease usually manifests only within 4 to 5 years in white children,[20] thus rendering it quite unlikely in the age range of our study population.

Our operational definition of failure to thrive by a weight percentile below 3% was too unspecific as it will predictably result in several findings in a sample of 260 children. Altogether, SAEs were rare and tended to be less common in the intervention group.

Beyond the already discussed shortcomings, the MARTHA trial is limited in its generalizability due to the selection of the study population, which does not represent a general population in terms of distribution of ethnicities, socioeconomic status, and family history, which are important determinants of allergic disease.[22,23]

We have focused on global markers of healthy development such as body weight and height, thereby we might have missed less obvious adverse effects such as iron deficiency, which could arise from the replacement of meat with milk as protein source. However, this is rather unlikely because the daily milk dose did not exceed the national recommendations and we discouraged additional milk feeding. Apart from the intervention milk, feeding was ad libitum thus allowing for sufficient meat consumption.

Taken together, the MARTHA study demonstrated the efficacy of weekly diaries and proved feasibility and safety of a cow's milk intervention in 6- to 36-month-old children. Close contact with participants was maintained by an electronic data capture system and frequent phone calls resulting in a low attrition rate. These instruments can easily be applied to larger studies as needed for definitive proof of the efficacy of minimally processed cow's milk for the prevention of respiratory infections, asthma, and allergies.

AUTHOR CONTRIBUTIONS

Melanie Weber: Formal analysis; methodology; data curation; validation; writing – original draft; writing – review and editing; visualization. **Franziska Hehn:** Formal analysis; methodology; investigation; writing – review and editing; visualization. **Yvi Huynh:** Formal analysis; investigation; writing – review and editing; data curation. **Aaron Remkes:** Writing – review and editing; software; investigation. **Christine Strunz-Lehner:** Writing – review and editing; conceptualization; methodology; project administration. **Irmgard Häuser:** Project administration; writing – review and editing; methodology. **Stefanie Hollunder:** Writing – review and editing; methodology; investigation. **Sheena Sharma:** Writing – review and editing; investigation; methodology. **Sibylle Contento:** Investigation; methodology; writing – review and editing. **Ulrich Mansmann:** Writing – review and editing; conceptualization; methodology; supervision; investigation; resources. **Erika von Mutius:** Investigation; funding acquisition; writing – review and editing; conceptualization; methodology; supervision; resources. **Markus Johannes Ege:** Supervision; writing – review and editing; conceptualization; methodology; investigation; funding acquisition.

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Research Conferences; Socieded Chilena de Enfermedades Respiratorias; Arla; Universität Leiden; OM Pharma S.A.; American Academy of Allergy, Asthma & Clinical Immunology; Deutsche Forschungsgemeinschaft (DFG); European Respiratory Society (ERS); Deutsche Gesellschaft für Kinder- und Jugendmedizin; World Allergy Organization (WAO); European Parliament; Gesellschaft für Pädiatrische Pneumologie (GPP); Helmholtz Association of German Research Centres; International Balzan Foundation "Prize." Patents planned, issued or pending: EvM has patent No. PCT/EP2019/085016 (Barn dust extract for the prevention and treatment of diseases) pending (Barn dust extract for the prevention and treatment of diseases) pending, royalties paid to ProtectImmun for patent EP2361632 (Specific environmental bacteria for the protection from and/or the treatment of allergic, chronic inflammatory and/or autoimmune disorders, granted on 19 March 2014), and patents EP1411977 (Composition containing bacterial antigens used for the prophylaxis and the treatment of allergic diseases, granted on 18 April 2007), EP1637147 (Stable dust extract for allergy protection, granted on 10 December 2008), and EP 1964570 (Pharmaceutical compound to protect against allergies and inflammatory diseases, granted on 21 November 2012) licensed to ProtectImmun.; Patent EP21189353.2. 2021. von Mutius E, Rankl B, Bracher F, Müller C, Walker A, Hauck SM, Merl-Pham J, inventors; PROTEINS IDENTIFIED FROM BARN DUST EXTRACT FOR THE PREVENTION AND TREATMENT OF DISEASES. Patent PCT/US2021/016918. 2021. Martinez FD, Vercelli D, Snyder SA, von Mutius E, Pivniouk V, Marques dos Santos M, inventors; THERAPEUTIC FRACTIONS AND PROTEINS FROM ASTHMA-PROTECTIVE FARM DUST; Patent EP21189353.2.2021. von Mutius E, Rankl B, Bracher F, Müller C, Walker A, Hauck SM, Merl-Pham J, Adler H, Yildirim A.Ö., Sattler M, Santos Dias Mourao A, Borggräfe J, O'Connor P.D., Plettenburg O, inventors; PROTEINS IDENTIFIED FROM BARN DUST EXTRACT FOR THE PREVENTION AND TREATMENT OF DISEASES; Participation on a Data Safety Monitoring Board or Advisory Board: Member of the EXPANSE (funded by European Commission) Scientific Advisory Board; Member of the BEAMS External Scientific Advisory Board (ESAB); Member of the Editorial Board of "The Journal of Allergy and Clinical Immunology: In Practice"; Member of the Scientific Advisory Board of the Children's Respiratory and Environmental Workgroup (CREW); Member of the International Scientific & Societal Advisory Board (ISSAB) of Utrecht Life Sciences (ULS) University of Utrecht; Member of External Review Panel of the Faculty of Veterinary Science, University of Utrecht; Member of the Selection Committee for the Gottfried Wilhelm Leibniz Programme (DFG); Member of the International Advisory Board of Asthma UK Centre for Applied Research (AUKCAR); Member of the International Advisory Board of "The Lancet Respiratory Medicine"; Member of the Scientific Advisory Board of the CHILD (Canadian Healthy Infant Longitudinal Development) study, McMaster University, Hamilton, Canada; Asthma UK Centre for Applied Research; Pediatric Scientific Advisory Board Iceland; Abbott Allergy Risk Reduction Advisory Board. Dr. Ege reports: Friesland Campina (milk powder for pilot phase); Dutch Longfonds Patient Organization (research funding); International Milk Genomics Consortium (travel grant).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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