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The Use of a Novel Donkey Milk-Derived Human Milk Fortified in the Neonatal Period Had No Effect on the Frequency of Allergic Manifestations During the First Years of Life: The "Fortilat Trial" Follow-Up

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Abstract

Background: Since human milk contents does not meet the high need of very low birth weight infants, fortification of breast milk is a standard practice for this population. As donkey milk has been long considered for children allergic to cow's milk proteins due to its low allergic properties, a new donkey milk-derived fortifier (DF) has been recently evaluated as a valid alternative to bovine milk-derived fortifier (BF). It seems to improve feeding tolerance when compared with standard BF, with similar neurodevelopmental and auxological outcome at 18 months of age. The aim of this study is to evaluate the development of allergic manifestations occurring in the population of the ''Fortilat Trial'' at 6–8 years of age.
Methods: Allergic manifestations were assessed by an ad hoc questionnaire administered to families. The occurrence of asthma, allergic rhinitis and oculorhinitis, rashes and atopic dermatitis, food allergies, accesses to an emergency department for allergic reactions, and the need of antihistamine have been investigated.
Results: In total, 113 infants were enrolled in the study (BF arm: $n = 60$, DF arm: $n = 53$). No difference in risk was observed between the two groups for all the considered outcomes. In conclusion, our data suggest that DF does not impact the development of allergic manifestations in the first years of life.
Clinical Trial Registration number: ISRCT N70022881

Keywords: human milk fortifier, donkey milk, adjustable fortification, VLBWi, preterm infants, allergy

Introduction

Literature data clearly indicate that human milk (HM) is the best source of nutrition for both term and preterm infants conferring health benefits both in the short and long term.[1] HM alone, however, does not meet the recommended nutritional needs for growth in very low birth weight infants (VLBWi) when given at the standard feeding volume, and nutrient fortification of HM is necessary.[2–5] Most multinutrient fortifiers are bovine milk derived, whereas recently, a new donkey milk-derived fortifier (DF) has been suggested as a valid alternative as HM fortifier.[6]

Donkey milk (DM) has been proposed for children allergic to cow's milk proteins for its hypoallergenic features.[7,8] Moreover, our group observed that DM is rather more similar (in terms of quantitative composition) to HM than the bovine milk.[9,10]

The randomized controlled trial named ''Fortilat'' performed in the NICU of the University of Turin compared the use of bovine milk-derived fortifier (BF) and DF in a cohort of preterm babies.[6]

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The aim of this part of the study is to extend our findings regarding the use of DM while evaluating the allergic manifestations in the first years of life of the infants enrolled in the clinical trial named ''Fortilat.'' Our hypothesis is that feeding premature newborns with HM fortified by a DF may impact the development of allergic manifestations in the first years of life, in comparison with an analogous population fed with traditional BF.

Subjects and Methods

Fortilat clinical trial

The study was performed in the neonatal intensive care unit of the University of Turin. It was approved by the local ethic committee (AN: 0025847, 27/05/2014) and registered (ISRCTN70022881). Between November 2014 and December 2016, 157 preterm babies with gestational age <32 weeks or birthweight ≤1,500 g were recruited.

Infants were randomized 1:1 in one of the following groups: the control group (BF arm) underwent adjustable (ADJ) fortification with a multicomponent fortifier and a protein concentrate derived from bovine milk; the Fortilat group (DF arm) underwent ADJ fortification with a multicomponent fortifier and a protein concentrate derived from DM. Refer to our previous articles for description of the study protocol, including characteristics of the protein supplements used in the trial, and results.[6,11–13]

Fortilat follow-up

Infants enrolled in the ''Fortilat'' trial were eligible in our study, which aims to investigate the development of allergic manifestations in the first 6–8 years of life.

A questionnaire including 25 items was administered to parents by two interviewers (Supplementary Appendix A1), after adequate training that included a phase of standardization of the answers reported by the 2 investigators through interviews simulation: 16 anonymous families not belonging to the trial were enrolled in the simulation conducted by both interviewers; a correspondence analysis was carried out by our biostatistical collaborator to evaluate responses' homogeneity and obtain adequate standardization. The 157 children enrolled in the ''Fortilat'' trial were randomized to interviewer 1 and interviewer 2; interviewers were unaware of the arm the children belonged to.

Clinical outcomes. Allergic manifestations assessed by the questionnaire included:

- Asthma
- Allergic rhinitis and oculorhinitis
- Skin rashes and atopic dermatitis
- Food allergies
- Access to an emergency department for allergic reactions
- Need of antihistamine.

The interviewers were trained to register only confirmed diagnosis. Regarding asthma episodes, only patients above the age of 5 years who had received a documented medical diagnosis were considered. This approach was extended to all the other investigated medical conditions: exclusively parents reported medical illnesses were discarded and only diagnosis ascertained by a pediatrician or a specialist was considered. Moreover, regarding the use of antihistamines, only the use associated with allergic reactions was reported. Interviewers had the opportunity to consult with each other in case of doubful reporting.

Sample size and rate of loss to follow-up. Of the 157 children, 41 children (26%) did not complete the follow-up for different reasons (early discharge, referral to local hospital), but were included in our trial since we hypothesize that any contact with DM proteins deserved to be investigated to evaluate the occurrence of allergic manifestations.

Statistical analysis

In the description of the sample, the categorical variables were presented as frequencies (percent), wheres the continuous variables were presented as mean (standard deviation) or median (interquartile range) according to their distribution.

Table 1. Family's Characteristics and Baseline Characteristics of Children Included in the Study

	BF arm n=*60*	*DF arm* n=*53*
Questionnaire answered by mother	49 (81.7)	42 (79.3)
Non-Italian mothers	18 (30.0)	8 (15.1)
Maternal education		
High school diploma	21 (35.0)	21 (39.6)
Degree	25 (41.7)	21 (39.6)
Smoke during pregnancy		
Ever	12 (20.0)	13 (24.5)
Beyond the 3rd month	6 (10.0)	5 (9.4)
Allergic parents		
Mothers	23 (38.3)	20 (37.7)
Fathers	18 (30.0)	17 (32.1)
Both	9 (15.0)	8 (15.1)
Family pets		
Ever	36 (60.0)	31 (58.5)
In the first year of life	25 (41.7)	25 (47.2)
Human milk		
Ever	60 (100.0)	52 (98.1)
At least 6 months	12 (20.0)	15 (28.3)
Interviewer 1	30 (50.5)	21 (39.6)
Girls	26 (43.3)	29 (54.7)
Gestational age, median (IQR)	29 (27.5;31)	31 (29;32)
Birth weight (*z*-score)	−0.72 (1.21)	−1.10 (1.27)
Birth length (*z*-score)	−1.03 (0.94)	−1.19 (1.44)
Large for gestational age[a]	2 (3.3)	1 (1.9)
Small for gestational age[a]	21 (35.0)	23 (43.4)
Age at interview (years), median (IQR)	6.7 (6.2;7.1)	6.7 (6.3;7.2)
Weight at interview[b] (*z*-score)	−0.96 (1.13)	−1.10 (1.18)
Height at interview[b] (*z*-score)	−0.47 (0.94)	−0.63 (1.13)

Data are summarized as *n* (%) if not otherwise specified.
[a]Reference: INeS charts.[14]
[b]Reference: SIEDP2006.[15]
BF, bovine milk-derived fortifier; DF, donkey milk-derived fortifier; IQR, interquartile range.

Table 2. Row Risk and Relative Risk (95% Confidence Intervals) of Allergic Outcomes

	BF arm RR (95% CI)	*DF arm RR (95% CI)*	*BF versus DF RR (95% CI)*	p
Wheezing	0.17 (0.09;0.29)	0.23 (0.14;0.37)	1.36 (0.64;2.89)	0.43
Nocturnal cough in the past 12 months	0.07 (0.03;0.17)	0.09 (0.04;0.22)	1.41 (0.40;5.00)	0.59
Asthma or asthmatic bronchitis	0.15 (0.08;0.27)	0.23 (0.14;0.37)	1.51 (0.69;3.30)	0.31
Use of drugs for asthma in the past 12 months	0.13 (0.07;0.25)	0.13 (0.07;0.26)	0.99 (0.39;2.55)	0.98
Access to emergency department for asthma	0.20 (0.12;0.33)	0.23 (0.14;0.37)	1.13 (0.56;2.30)	0.73
Frequent sneezing or rhinitis	0.13 (0.07;0.25)	0.09 (0.04;0.22)	0.71 (0.25;2.03)	0.52
Itchy skin rash and dry skin	0.25 (0.16;0.39)	0.26 (0.17;0.41)	1.06 (0.56;1.98)	0.86
Food allergen reaction	13.8 (0.07;0.26)	13.2 (0.07;0.26)	0.96 (0.37;2.46)	0.93
Use of antihistamine drugs	0.27 (0.17;0.41)	0.24 (0.15;0.39)	0.92 (0.49;1.73)	0.79

CI, confidence interval; RR, relative risk.

The row risk of occurance for each outcome was estimated using Poisson regression with robust error variance, and the relative risk (RR) was used to compare the arms, using BF arm as reference.

Given the impossibility of assuming a random distribution of the losses to follow-up, in a second step analysis, adjusted risks were estimated using the same procedure.

SAS, version 9.4 (SAS Institute, Inc.), was used to process data and fit statistical models.

Results

The flow chart for the enrollment of the study population is available (Supplementary Appendix A2).

In total, 157children were eligible : 79 of them were randomized to interviewer 1 and 78 babies to interviewer 2. A total of 44 babies (28% of the whole population) were lost to follow-up; the final sample was composed of 113 babies, 60 of whom (53%) were from the BF arm (30 to interviewer 1 and 30 to interviewer 2) and 53 (47%) were from the DF arm (21 to interviewer 1 and 32 to interviewer 2).

Children's family and baseline characteristics

The distribution of the children's family and baseline characteristics is described (Table 1). Among children whose mothers presented an history of allergic issues, food allergies were reported in eight cases both in the BF arm and the DF arm, respectively, 35% and 40% of the allergic mothers, whereas asthma was reported in 7 mothers out of 23 (30%) in the BF arm and 6 mothers out of 20 (30%) in the DF arm. Regarding children whose fathers had an history of allergic issues, food allergies were reported in two cases in the BF arm and three cases in the DF arm, respectively, 11% and 18% of the allergic fathers; asthma was reported in 6 out of 18 cases (33%) in the BF arm and 6 out of 17 cases (35%) in the DF arm.

In the DF arm, the mean birthweight *z*-score was lower, and the percentage of small for gestational age was slightly higher, as is the weight *z*-score at interview, compared with the BF arm. The age at interview was very similar.

Allergic diseases

Row risk and RR (Table 2) and adjusted RR (Table 3) for allergic diseases and associated issues are reported in detail.

Wheezing or nocturnal coughing

No significant differences emerged regarding the risk of presenting at least one episode of wheezing since birth or nocturnal cough in the past 12 months.

Asthma or asthmatic bronchitis

No difference emerged in the risk of asthma or asthmatic bronchitis, as well as for the use of drugs against asthma in the past 12 months, and access to an emergency department for asthma or asthmatic bronchitis.

Sneezing and rhinitis

The RR of frequent sneezing and runny nose not associated with flu or cold did not differ significantly. Medical diagnosis of allergic rhinitis occurred in two (3%) and one (2%) case in BF arm and DF arm, respectively.

Itchy rashes and atopic dermatitis

The risk of itchy skin rash and dry skin was very similar. A diagnosis of atopic dermatitis or eczema was made by a physician for eight (13%) and three (6%) children in the BF arm and the DF arm, respectively.

Table 3. Relative Risk (95% Confidence Intervals) of Allergic Outcomes Adjusted by Gender, Birth Weight (*z* Score), Gestational Age, Human Milk at Least for 6 Months of Life, Maternal Italian Nationality, Interviewer

	BF versus DF RR_{adj} (95% CI)	p
Chest wheezing or whistling	1.33 (0.63;2.82)	0.46
Night cough in the past 12 months	1.43 (0.32;6.34)	0.64
Asthma or asthmatic bronchitis	1.45 (0.67;3.15)	0.34
Use of drugs against asthma in the past 12 months	1.04 (0.41;2.59)	0.94
Access in emergency department for asthma	1.09 (0.52;2.27)	0.83
Frequent sneezing or rhinitis	0.51 (0.19;1.36)	0.18
Itchy skin rash and dry skin	0.88 (0.47;1.66)	0.70
Food allergen reaction	0.84 (0.35;2.01)	0.69
Use of antihistamine drugs	0.85 (0.43;1.67)	0.64

Allergic reactions to foods

The risk of the occurrence of at least one allergic reaction to foods was similar in the two arms as was the risk of use of antihistamine drugs. Among the 15 children having an allergic reaction to foods, only 1 out of 8 (12.5%) in the BF arm and 2 out of 7 (29%) in the DF arm had a medical diagnosis. Among the 113 children, 2 (3%) in the BF arm and 6 (11%) in the DF arm had an access to an emergency department because of an allergic reaction.

Adjusted risk and RR

Many variables were associated with the outcomes, some are children's characteristics (i.e., gender, birth weight as *z*-score, gestational age, HM for at least 6 months, and age at interview), others are parent's features (i.e., maternal education, maternal smoke during pregnancy, maternal nationality, and parents' history of allergies).

Among these variables, we decided to include in a model to correct our results, those with a different distribution between the two arms, independently of their statistical significance: gender, birth weight as *z*-score, gestational age, HM for at least 6 months, and maternal nationality. Furthermore, we considered the interviewer as covariate. The adjusted RRs and their 95% confidence interval are reported in Table 3: as for row RR, no differences between the two arms emerged.

Discussion

In this study, we wanted to investigate whether the introduction of a DM-derived HM fortifier could play any role in the development of allergic manifestations. Moreover, since sensitization can occur very early in life, we supposed that early factors during lactation may have a role in preventing allergies.

Literature suggests that premature babies are likely to have enhanced exposure to antigens and thus may be expected to be at major risk of sensitization and development of allergic disease. In infancy, the main allergic symptoms are atopic dermatitis, gastrointestinal symptoms, and recurrent wheezing, whereas bronchial asthma and allergic rhinoconjunctivitis appear later in childhood. Food allergies, such as cow's milk protein allergy, are most common in the first year of life, whereas allergy to inhalant allergens mostly occurs later.

Breastfeeding seems to have a protective role against the development of recurrent wheezing and asthma,[16] but many factors, such as structural airways abnormalities, immune response to viral pathogens or aeroallergens, inflammation, and microbiome diversity, may play a pathogenetic role.[17]

The association between food allergies and prematurity is more debated. Premature babies may experience increased intestinal permeability and increased food antigen uptake, which can lead to an increased risk of IgE-mediated allergy. However, findings from other studies have questioned the role of prematurity and LBW in the development of food allergy.[18–21]

Another study reported that early avoidance of cows' milk proteins in neonates does not protect against later atopy or sensitivity to food.[22]

The risk of developing allergic diseases due to exposure to allergens such as cow's milk protein is unknown among very preterm infants. An randomized controlled trial (RCT) showed that BF or preterm formula for preterm infants did not increase the risk of developing allergic symptoms.[23]

Milk from other mammalian sources than the cow has received some attention for its different protein composition profile that can result in a potentially low cross-reactivity with cow's milk proteins. Among these, DM has been proposed for feeding cow's milk allergic patients. Literature showed that DM was better tolerated in patients with atopic dermatitis and cow's milk allergy.[7,8,24]

Our results showed that DF does not seem to have a protective effect on occurance of allergic manifestations such as asthma, skin rashes, food allergies and number of accesses to an emergency department for allergic reactions. As expected, results suggest that having a parent suffering from allergies increases the patient's risk of developing food allergies. The number of cases of atopic dermatitis was too small to allow for a significant analysis.

Our data reflected our expectation: our aim was to show no differences in children's risk of developing allergic isssues when exposed to a new DF. However, this study is grafted into an RCT planned for another purpose, therefore, it was not possible to plan the number of samples according to our results, and the absence of differences could simply be due to low power. Nevertheless, it should be noted that studies of this type are still lacking and ours can be useful for planning future research designed as no inferiority or superiority studies.

Conclusions

In conclusion, our data reflected no differences in the use of BF compared with the use of DF as regard the risk of allergic manifestations. However, for its benefits on the feeding tolerance in a particularly frail population such as the very preterm and VLBWi population, we suggest that the new DF should be used as a new fortifier for premature patients in NICUs.

Authors' Contributions

Conceptualization was carried out by E.S., S.D., M.B., L.C., M.G., I.C., G.E.M., C.P., and A.C.; investigation was done by S.D., M.B., and M.A.; methodology was carried out by E.S.; supervision was by C.P. and M.A.; writing—original draft was by S.D., C.P., and E.S.; and writing—review and editing was done by S.D., E.S., L.C., M.G., G.E.M., F.C., C.P., and A.C. All authors have read and agreed to the published version of the article.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and it was approved by local ethic committee (AN: 0025847, 27/05/2014) and registered (ISRCTN70022881).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Disclosure Statement

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Supplementary Material

Supplementary Appendix A1
Supplementary Appendix A2

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