Nov 12, 2025 Version 1

Evaluating the Long-Term Impact of a Partially Hydrolyzed Formula on Allergy Prevention in High-Risk Infants: The Allergy Reduction Trial (A.R.T.) 5-year Follow-up Protocol V.1

DOI
https://dx.doi.org/10.17504/protocols.io.kxygx4bezl8j/v1

Ruzha Pancheva[1], Nicolaos Nicolaou[2], Mikaela Sekkidou[3], Eva Karaglani[4], Simoneta Popova[5], Paris Kantaras[6], Theodora Boutsioukou[7], Zoi Iliodromiti[8], Nicoletta Iacovidou[9], Vassiliki Papaevangelou[6], Linde van Lee[7], Yannis Manios[4]

[1]Department of Hygiene and Epidemiology, Faculty of Public Health, Medical University of Varna, *Prof. Dr. Paraskev Stoyanov* Bulgaria;
[2]Asthma and Allergy Centre, Limassol, Cyprus; [3]University of Nicosia Medical School, Nicosia, Cyprus;
[4]Department of Nutrition 26 Dietetics, School of Health Science 26 Education, Harokopio University, 17671, Athens, Greece;
[5]Neonatal Department, National and Kapodistrian University of Athens, Aretaieio Hospital, Athens, Greece;
[6]Third Department of Pediatrics, National and Kapodistrian University of Athens, ATTIKON General University Hospital, Athens, Greece;
[7]FrieslandCampina, Amersfoort, the Netherlands;
[8]European Centre for Obesity Research, Harokopio University, 17671 Athens, Greece;
[9]Institute of Agri-food and Life Sciences, Hellenic Mediterranean University Research Centre, Crete, Greece

Mirre.Viskaal

DOI: https://dx.doi.org/10.17504/protocols.io.kxygx4bezl8j/v1

Protocol Citation: Ruzha Pancheva, Nicolaos Nicolaou, Mikaela Sekkidou, Eva Karaglani, Simoneta Popova, Paris Kantaras, Theodora Boutsioukou, Zoi Iliodromiti, Nicoletta Iacovidou, Vassiliki Papaevangelou, Linde van Lee, Yannis Manios 2025. Evaluating the Long-Term Impact of a Partially Hydrolyzed Formula on Allergy Prevention in High-Risk Infants: The Allergy Reduction Trial (A.R.T.) 5-year Follow-up Protocol. **protocols.io https://dx.doi.org/10.17504/protocols.io.kxygx4bezl8j/v1**

Protocol status: Working
We use this protocol and it's working

Created: November 12, 2025

Last Modified: November 12, 2025

Protocol Integer ID: 232180

Keywords: high-risk infants, partially hydrolyzed formula, food allergies, atopic dermatitis, long term allergy prevention, growth, nutritional interventions on allergy development, hydrolyzed formula on allergy prevention, milk protein allergy, long term allergy prevention, refining infant feeding recommendation, allergy reduction trial, phf on allergy prevention, infant feeding recommendation, nutritional intervention, allergy prevention, allergy development, risk infant, food allergy, allergy, retrospective data on allergic manifestation, associations between feeding type, whey, hydrolyzed formula, analysis for the primary outcome, infant, including smoking exposure, feeding type

Abstract

Introduction:

Cow's milk protein allergy (CMPA) affects 2–5% of infants. However, partially hydrolyzed formulas (pHF) are considered a potential alternative for high-risk infants who cannot be exclusively breastfed. The Allergy Reduction Trial (A.R.T.) previously assessed the short-term effects of a whey-based pHF on allergy prevention in high-risk infants. This 5-year follow-up study evaluates the long-term impact of early nutritional interventions on allergy development and growth outcomes.

Methods and Analysis:

A.R.T. is a multicenter double-blinded randomized controlled trial in Bulgaria, Greece, and Cyprus. Participants were initially enrolled as newborns (2017–2019) and randomized to receive either a pHF, or a standard formula (SF) in case exclusive breastfeeding (EBF) was not feasible. A group of EBF, was also followed-up as an observational group. The 5-year assessment consists of a single visit or phone interview, collecting retrospective data on allergic manifestations (e.g., atopic dermatitis, food allergies, respiratory allergies) and growth parameters (weight, height, BMI) through parental questionnaires and medical records. Additional demographic data were obtained including smoking exposure, daycare attendance, and other environmental factors.

Statistical analysis will employ generalized estimating equations (GEE) to assess associations between feeding type and allergy development, adjusting for potential confounders. Growth outcomes will be analyzed using mixed-effects models. Data will be evaluated on an intention-to-treat (ITT) basis, and per-protocol (PP) analysis for the primary outcomes.

Discussion:

This study provides critical long-term data on early-life nutritional interventions and allergy prevention. Findings will contribute to refining infant feeding recommendations and informing clinical guidelines for high-risk populations.

Trial Registration:

Registered at ClinicalTrials.gov (NCT05418491 and NTR6259)

Key words

high-risk infants, partially hydrolyzed formula, food allergies, atopic dermatitis, long term allergy prevention, growth

Troubleshooting

3. Discussion

1 1.1. **Background and rationale**

Cow's milk protein allergy (CMPA) is an increasing healthcare problem during infancy, caused by an abnormal immune response to cow's milk proteins [1]. CMPA affects approximately 2-5% of newborns within the first year of life, with around 60% of these cases being IgE-mediated [2]. In general, about 5-15% of infants display symptoms suggestive of CMPA[3]. AD is the most common skin disease in childhood, with over 80% of cases developing before the age of 5, especially during the first 6 months of life [4].

While breastfeeding is considered optimal for infant nutrition and may lower the overall risk of allergic diseases, it should not be regarded as the most effective preventive strategy for cow's milk protein allergy, as cow's milk proteins can be transferred through breast milk and trigger hypersensitivity reactions in predisposed infants [5], [6], [7], [8]. Indeed, the American College of Allergy, Asthma & Immunology recommends exclusive breastfeeding for the first 4 to 6 months of life as a preventive measure against food allergies, including cow's milk protein allergy, although the strength of this recommendation is weak and the evidence is graded as C [9]. Similarly, the National Institute of Allergy and Infectious Diseases (NIAID) guidelines also recommend exclusive breastfeeding until 4 to 6 months of age for all infants, including those with a family history of atopic disease, due to the general health benefits of breastfeeding, despite the unclear evidence regarding its protective role against atopic diseases [10].

The current consensus in the medical literature supports the notion that early introduction of allergenic foods can decrease the risk of developing food allergies [11]. The National Institute of Allergy and Infectious Diseases (NIAID)-sponsored Expert Panel suggests that the use of hydrolyzed infant formulas, as opposed to cow's milk formula, may be considered as a strategy for preventing the development of food allergy in at-risk infants who are not exclusively breastfed [12], although recommendations vary [13]. The effectiveness of partially hydrolyzed formulas (pHFs) in reducing the risk of developing allergic symptoms during infancy, particularly when breastfeeding is not feasible, has recently come under scrutiny. The effectiveness of pHFs in reducing the risk of developing allergic symptoms during infancy is evaluated in several studies with mixed results. Earlier studies by von Berg et al. (2003) [14][11] and Hays & Wood

(2005) [15]
show that formulas with hydrolyzed protein fractions, offer strong protection against developing AD. A meta-analysis concludes that there is no consistent evidence supporting the use of pHFs to reduce allergy risk[16]. Another, more recent systematic review
and meta-analysis by Li et al. (2024) [17] finds moderate evidence that pHF reduces the risk of eczema in children younger than 2 years (RR: 0.71; 95% CI: 0.52, 0.96) and wheeze at age 0-2 years compared with cow's milk formula (CMF) (RR: 0.50; 95% CI: 0.29, 0.85). Although the risk reduction is less than that observed with extensively hydrolyzed proteins (RR: 0.79; 95% CI: 0.67, 0.94 for eczema), pHFs have potential advantages, such as a better taste and lower price. However, the preventive efficacy of pHFs must be established on a product-by-product basis. Given the high prevalence of allergic manifestations, particularly among high-risk infants, and the mixed evidence regarding the efficacy of pHFs in reducing allergy risk, it is critical to further investigate.
In the present 5-year follow-up assessment,
the potential preventive role of a nutritional intervention within the first 6 months of life with a specific pHF compared to a standard cow's milk formula (SF) and exclusive breastfeeding on the development of allergic diseases up to the age of 5 years is investigated.

2 **Overview of the Clinical Trial**1.2.1. The Allergy Reduction Trial (A.R.T.) initial assessments
A.R.T. was a multicenter study
designed to evaluate the effectiveness of a specific nutritional intervention in reducing the risk of developing allergic manifestations, specifically Atopic Dermatitis (AD) and CMPA, in infants at high risk for allergies (based on a confirmed by a doctor family history of allergy in a first degree relative).
The study involved a partially hydrolyzed formula (pHF; Frisolac Gold HA) as a nutritional
intervention strategy against the development of allergies, compared to a standard formula (SF; Frisolac Gold). In addition, high risk for allergy infants who were exclusively breastfed were also followed-up as an observational group. This study followed the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2013 guidelines to ensure the highest standards in trial design, implementation, and reporting[18].
Between 2017 and 2019, healthy term
newborns at high risk for allergy were recruited within 4 days after delivery in 6 centres in 3 countries; Bulgaria (1), Cyprus (1) and Greece (4) for the A.R.T. birth cohort study. Non-exclusively breastfed infants were randomized into two groups—those fed with pHF and those fed with SF (either exclusively or in combination with breastfeeding). Exclusively breastfed infants were followed-up

as an observational group. Detailed description of screening and recruitment has been provided elsewhere [19]. The A.R.T. study focused on the prevention of allergic manifestations, particularly AD and CMPA during the first six months of life. Allergic outcomes such as AD and CMPA, were objectively assessed along with growth parameters that were carefully monitored during this period. At 1 year an additional questionnaire - The Comprehensive Early Childhood Allergy Questionnaire adapted [20] designed for detecting AD, asthma, and
IgE-mediated food allergies in children aged 1–5 years was completed in referring to the period of 6 months to 1 year.

1.2.2. Follow-up Assessment:

An additional assessment is undertaken at the age of five years. This assessment aims to evaluate the sustained impact of early nutritional intervention on the development of allergies and growth outcomes over the period of 1 to 5 years. The occurrence of any allergic condition in this period, including food allergies, respiratory allergies, and other allergic manifestations, are documented through a questionnaire based on parental reporting and doctor diagnosis.

3 **1.3. A.R.T Initial Objectives and First Results**

The primary objective of the original A.R.T. trial was to investigate the risk-reduction effect of a whey-based pHF compared to SF on the development of cow's milk protein allergy (CMPA) and atopic dermatitis (AD) in infants at high-risk for allergy within the first six months of life. Published results showed that of 331 randomized subjects (ITT analysis set), 160 received the pHF and 171 the SF. Six (3.8%) infants in the pHF and 12 (7%) in the SF group developed CMPA (p=0.186) diagnosed by CMP elimination diet followed by an Oral Food Challenge. AD incidence was significantly lower in those receiving pHF as compared to SF (10.6% vs. 18.7%, p=0.024) with a relative risk (RR, 95% CI) of 0.54 (0.32, 0.92), in particular when stratifying for family history of AD [6.5% vs. 27.3%, RR 0.24 (0.07, 0.78), p=0.018] representing a risk reduction of 76%. The PP analysis showed similar results[19].
The secondary objective was to compare growth parameters, including weight, height, and BMI, between infants fed with the pHF and those fed with the SF. Published results showed that growth did not differ between the aforementioned groups [21].
A tertiary objective was to examine the relationship between early life infections (ELIs), type of nutrition, and allergic manifestations in the first 6 months. Results showed a significantly lower risk of CMPA in infants with ELIs (3% vs. 13.4%, p = 0.001), with no cases observed in EBF infants who experienced ELIs. A trend toward reduced AD incidence was noted in infants fed with pHF (6.5%) compared to SF (18.2%, p = 0.092) [22].

4 **Protocol for the 5-year Follow-Up Assessment**

2.1. Objectives

The 5-year follow-up assessment aims
to evaluate the long-term effects of early nutritional interventions on
allergic manifestations and growth outcomes in children up to 5 years of age.

2.2 Study Design

The follow-up assessment is structured as an observational study with a single follow-up visit when the child reaches 5 years of age (+ 3 months). During this visit, retrospective information is collected regarding allergic symptoms, allergies diagnosed by a doctor and, growth parameters for the years 1, 2, 3, and 4, as well as updated demographic data. At the visit, height and weight measurements are recorded, while for those followed-up by phone, these data are collected from their medical records.

2.3. Methods: participants, outcomes

2.3.1. Study Setting

The study is a multicenter trial conducted in Greece, Bulgaria, and Cyprus. Participants are recruited from the original cohort of the A.R.T. study, which was conducted in these regions. Each site is responsible for organizing and conducting the 5-year follow-up visit, during which demographic data, anthropometric measurements, allergic manifestations and diagnoses are collected. The participating centers include Harokopio University in Greece (Ethical approval G-1296/24-03-2022), Medical University of Varna in Bulgaria (Medical ethical committee approval 115/31.03.2022), and Asthma and Allergy Centre in Cyprus (Cyprus Bioethics Committee approval ΕΕΒΚ ΕΠ 2022.01.159). If in-person visits are not feasible, follow-ups are conducted by phone.

2.3.2. Eligibility Criteria for the 5-year Follow-Up

Children eligible for the follow-up assessment must have been part of the Intention-to-Treat population of the A.R.T. study and fall within the age range of 5 years to 5 years and 3 months at the time of the follow-up. At least one parent or legal guardian must provide written consent. Children who did not participate in the A.R.T. study or who were not assigned to any of the three study arms or whose parents or guardians decline to provide consent are excluded.

2.3.3 Study Flow Diagram and Participant Inclusion

The flow diagram of the participants through each stage of the A.R.T. study and the 5-year follow-up is presented in Figure 1, following the CONSORT guidelines for observational follow-up studies.

2.3.4. Study parameters

Demographic Data Collection

Demographic information obtained during the initial assessment of the A.R.T. study is used for comparison across study groups. The following demographic variables are

considered:

- Country of birth of the child
- Gender
- Mode of delivery
- Age of the parents
- Parental education level

Additionally, families are asked to provide updated demographic data, including:

- Duration of breastfeeding (for mixed-fed and exclusively breastfed infants)
- Urban or rural place of residence
- Number of household residents
- Presence of pets at home
- Smoking and vaping habits of the parents
- Child's attendance at daycare
- Child's history of COVID-19 infection

Parents were allowed to introduce complementary (solid) foods after 17 weeks of age, in accordance with international feeding guidelines. The timing of solid food introduction was recorded through parental report and, when available, verified using medical records or child health booklets.

Assessment of Allergic Manifestations

The single visit at 5 years of age serves as the primary data collection point for the follow-up assessment. The presence of allergic manifestations in study participants between the age of one to five years is evaluated through an enriched questionnaire. This questionnaire is based on the validated ISAAC (International Study of Asthma and Allergies in Childhood) Questionnaire [23] and the Childhood Allergy Questionnaire [20]. The researchers ask the parents and/or legal guardians these questions and record their answers. The follow-up study captures data on various allergic conditions, including food allergies, respiratory allergies (asthma and rhinitis), eczema/atopic dermatitis, urticaria, drug allergy, and insect venom allergy. The collected data includes the presence of these conditions, doctor diagnosed at any time point between their child's first and fifth birthday as reported by parents and confirmed through medical records where available.

Anthropometric Measurements

Body Weight

Body weight is measured in kilograms using standardized digital scales (Seca) with a precision of ±100 g for weights under 150 kg. The scales are placed on a stable, flat surface to ensure accurate measurements. Trained study researchers ensure that children remain

stable during the procedure. Calibration of the weighing scale is performed regularly using a 5 kg weight standard. Children are weighed wearing only light clothes, without shoes, jewellery, or accessories. Measurements are taken in duplicate, and the average value is recorded. If there is a discrepancy of 100 grams or more between the two measurements, a third measurement was taken. The two values closest to each other were recorded and taken to calculate the average.

Body Height

Height is recorded in centimetres and measured to the nearest 0.1 cm using a height-measuring station (Seca). All children are measured without shoes, with untied hair and no hair accessories. The measuring station is placed on a flat, stable surface. Trained study researchers ensure that children maintain a stable posture during the measurement. Each measurement is taken twice, and the final average value is recorded.

If there is a significant discrepancy between the two measurements of 1 cm or more, a third measurement was performed. The average of the two measurements after excluding the one with the largest deviation was recorded.

If not measured on site, information on anthropometrics is retrieved from children's medical records via phone call. Retrospective growth data for the years 1, 2, 3, and 4 are also obtained from parental reports and medical records to complete the child's growth trajectory.

2.3.5. Sample Size Calculation:

No sample size was undertaken for the 5-year follow-up. Effort was taken to enrol as much eligible children from the A.R.T study.

2.3.6. Safety

The methods used during this 5-year follow-up assessment include a single visit and anthropometric measurements or a phone call, which are not invasive and hardly time consuming. Since there is no treatment used, that could lead to any (severe) adverse events, no procedures on safety reporting are needed.

5

2.3. Methods: data management and analysis.

2.3.1. Statistical Analysis Plan:

The same methodology in statistical analysis plan is followed as in the initial A.R.T. study. Since outcome in allergic manifestations (AMs) concerns count data, Poisson analysis will be applied. The outcomes for any AM (including atopic dermatitis, food allergy, allergic rhinitis, and asthma) and each individual AM will be analysed as obtained for

the ages 1-5 years and cumulatively since birth, taking into account the outcomes within the first 6 months of life and at the age of 12 months. All analyses for any AM as an outcome, will be performed using Generalized Estimating Equation (GEE) for binary outcomes. All analyses will be performed on Intention-to-Treat basis (ITT). A Per-Protocol population (PP) analysis will be conducted for the primary outcome (any AM). The PP population consists of the subjects that were compliant to the A.R.T. study protocol up to the age of 6 months and additionally are compliant to the A.R.T. 5-year follow-up protocol. In case of any protocol deviations occurred during A.R.T. or A.R.T. 5-year follow-up, these children will be included only in the ITT analysis set. Descriptive presentation of the outcomes in each study group will be shown, and relative risk with 95% Confidence Interval will be estimated applying two-sided testing.
Possible confounding factors such as
family history of allergies, smoking habits, daycare attendance, rural/urban residence, presence of pets at home, exposure to tobacco smoke/vape smoking, COVID-19
infection) will be evaluated. The variables which will show to have to a statistically significant effect on the outcome based on logistic regression analysis or based on the literature, will be considered as covariates in the analysis.
For the anthropometric outcomes, Body
Mass Index (BMI) will be calculated by weight
divided by squared height. Weight, height, and BMI will be transformed to age-and-sex adjusted WHO z-scores. All outcomes will be assessed for outliers using Grubbs' test for continuous data. Missing values will not be imputed. Descriptive presentation of the various outcome parameters will include mean, standard deviation, number of observations, standard error of the mean, and if appropriate median, interquartile range (IQR) and range. Growth outcomes for the ages 1 to 5 years will be analysed
to assess any differences between the groups and between the groups and the standard WHO growth charts.

2.3.2. Handling Longitudinal Data:

Although all data
were collected retrospectively between 1-year and 5-year visit, it will be analysed as if gathered longitudinally

6 **2.4. Methods: Combined Sections for Both A.R.T. Assessments**2.4.1. Data Management and Storage
Data are collected using both hard copy and
digital Case Report Forms (CRFs) via the Viedoc system (4Pharma), following a four-eyes

principle for data entry verification. Research assistants, trial coordinators, and project experts enter data weekly. Principal Investigators (PIs) or designated research assistants have access to Viedoc, while data monitoring is performed remotely by the sponsor. Any discrepancies are addressed immediately via email.

Study-related documents, including the study protocol, ethics approvals, reports, and communication records, are stored in a Trial Master File (TMF) at each study site. Informed Consent (IC) forms are securely stored at local investigator sites for 10 years post-study completion, while pseudonymized datasets are be preserved for 15 years at local study centres and FrieslandCampina.

2.4.2. Monitoring and Quality Assurance

Regular monitoring site visits ensures compliance with the study protocol. Monitoring activities include reviewing Informed Consent Forms, participant eligibility, recruitment procedures, and CRFs.

Database Lock

Upon completion of data collection, a Data Review Meeting (DRM) with the Trial Coordinator, Sponsor, and Data Collection Coordinator validates the dataset before locking it. All data remain pseudonymized using unique numeric codes in accordance with ICMJE guidelines for data sharing.

7 **2.5. Ethics and Dissemination**

2.5.1. Informed Consent Process

Informed consent is obtained in both the original A.R.T. study and the 5-year follow-up, ensuring that parents or guardians fully understand the study objectives, procedures, potential risks, and benefits. For the follow-up assessment, a new consent form is collected at the 5-year visit, either in person or remotely, to confirm voluntary participation.

2.5.2.Ethical Considerations

The study complies with the Declaration of Helsinki and GCP guidelines. Ethical approval is obtained from the respective ethics committees in each participating country. Additionally, the study is registered in the ClinicalTrials.gov database (https://www.clinicaltrials.gov/) before recruitment starts.

2.5.3. PublicDisclosure and Publication Policy

Following analysis, study results will be compiled into a report for FrieslandCampina and submitted for publication in an international peer-reviewed journal.

2.5.4. Funding

The A.R.T. trial is funded by FrieslandCampina, the manufacturer of the pHF and SF used, in collaboration with the research institutions involved in the study. The funding covers all aspects of the trial, including data collection, participant follow-up. The sponsors have no role in the direct execution of the study, and the trial is conducted independently by the research team to maintain scientific integrity and minimize bias. Full transparency regarding the funding and any potential conflicts of interest is ensured through adherence to international clinical trial reporting standards.

8 **2.**

Discussion

Rationale for a

Follow-up assessment

The need for a follow up assessment of the A.R.T. study is critical for several reasons:

By conducting a follow-up assessment at 5 years, the A.R.T. study captures both the short-term and prolonged effects of nutritional interventions, ensuring that recommendations for allergy prevention in high-risk infants are based on comprehensive, long-term evidence.

3.1. Context and Relevance of Findings

The findings from the A.R.T. study and 5-year follow-up contribute to a growing body of research exploring the long-term impact of early nutritional interventions on allergy prevention. The relationship between infant feeding practices and allergic disease development is dynamic, with factors such as immune maturation, genetic predisposition, and environmental influences playing critical roles. While the short-term effects of early-life feeding choices, including breastfeeding and hydrolyzed formulas, have been better documented, their long-term impact remains an area of even more needed investigation. The A.R.T. follow-up assessment provides valuable insights into whether the early benefits of nutritional interventions persist into childhood or whether their effects diminish over time.

3.2. The Need for Long-Term Follow-Up in Allergy Prevention

Long-term follow-up studies in allergy research are essential to distinguish between transient effects and sustained benefits. While breastfeeding has been shown to provide early protection against allergic conditions such as eczema, some studies suggest that these benefits may wane with age, leading to what has been termed a "rebound effect"[24]. This phenomenon underscores the need for extended follow-up periods to determine whether early protective effects remain stable or diminish over time. The A.R.T. study addresses this gap by tracking allergic manifestations up to five years of age, contributing to a deeper

understanding of the long-term trajectory of allergy risk following early-life dietary interventions.

The concept of allergy prevention has evolved significantly in recent years. Previous guidelines recommended delaying the introduction of allergenic foods to prevent allergies; however, contemporary research challenges this approach. Studies on peanut and egg introduction suggest that early exposure to allergenic foods reduces the risk of developing corresponding allergies [25], [26]. Despite these promising short-term findings, the long-term impact of early dietary interventions remains unclear. The A.R.T. study provides much-needed longitudinal data by evaluating whether an early intervention with a specific pHF influences allergy risk beyond infancy.

2.3. Variability in Infant Feeding and Allergy Outcomes

One of the challenges in understanding allergy development is the variability in feeding practices among infants. While some are exclusively breastfed, others receive breastmilk substitutes including SF and pHF, often in combination with breastfeeding. Research suggests that these different feeding approaches may be associated with varying risks of allergic symptoms, but the precise mechanisms remain uncertain [27]. Additionally, genetic predisposition, parental allergies, and prenatal exposures contribute to the complexity of allergic disease development. By incorporating long-term follow-up data, studies like A.R.T. help clarify these relationships and provide evidence to inform clinical recommendations.

Growth and Safety Parameters:

In addition to allergic outcomes, both the A.R.T. study and the 5-year follow-up assessment examines growth metrics (weight, height, BMI) to ensure the nutritional safety of the formula being tested. The long-term assessment is essential for determining whether any differences in growth emerge over time, providing critical data on both the efficacy and safety of the interventions.

2.5. Evidence on Long-Term Effects of pHF

Beyond the A.R.T. study, only a few studies have long-term assessed the safety and efficacy of hydrolyzed formulas in terms

of allergy prevention and growth..

3.5.1. Allergy

Prevention

The German Infant Nutritional Intervention (GINI) study one of the most comprehensive trials in this field followed children into adolescence also providing key insights into the role of hydrolyzed formulas in allergy prevention. At 10 years, children who had received pHF-W as infants showed a significantly lower cumulative incidence of allergic diseases, particularly atopic dermatitis, compared to those fed with a SF. This protective effect extended into adolescence, with a 15-year follow-up revealing a lower prevalence of allergic rhinitis and eczema among those who had been given pHF-W in infancy[28], [29] . These findings suggest that dietary modifications during early life may have lasting effects on immune system programming and allergy risk reduction. The A.R.T. follow-up study builds upon these observations by examining whether similar trends are observed in a different population, further strengthening the evidence supporting pHF as a potential allergy prevention strategy.

3.5.2. Growth Outcomes

The GINI study, also assessed whether early dietary interventions influenced long-term growth. Over a 10-year follow-up, no significant differences were observed in BMI trajectories between infants fed pHF, CMF, or those who were breastfed. The only notable finding was a transient slower BMI gain in the first year of life among infants who consumed extensively hydrolyzed casein formula (eHF-C), but this effect was not sustained [30]. These findings, which should be confirmed on a case-by-case basis in accordance with EU legislation, reinforce the conclusion that hydrolyzed formulas do not negatively impact long-term and can be considered safe from a nutritional standpoint.

3.6. Clinical Implications and Future Directions

The findings from the A.R.T. 5-year follow-up study, along with evidence from previous trials, contribute to the ongoing discussion about the role of hydrolyzed formulas in allergy prevention. While pHF appears to be a safe alternative to SF, particularly for infants at high risk for allergies, its efficacy as a preventive intervention remains a subject of debate. Differences in hydrolysis processes between formulas mean that not all pHF products may have the same effect, underscoring the need for product-specific evaluations.

Long-term follow-up studies are invaluable for shaping public health recommendations, but they also present challenges, including participant retention and the need to control for

multiple confounding factors. Ensuring rigorous methodology is essential to generating reliable data that can inform both population-level guidelines and individualized clinical decisions. The A.R.T. study contributes to this effort by providing robust follow-up data, offering insights that will help refine current strategies for allergy prevention and guide future research on early-life nutritional interventions.

3.7. Challenges and Considerations

***Retrospective Data Collection*:**

One of the primary challenges is the reliance on retrospective data collection for the 1-5 year period, gathered during the 5-year visit. This introduces the possibility of recall bias from parents, especially regarding allergic symptoms that may have occurred several years prior. To address this, parents were asked to review any available medical records, regarding growth parameters.

***Compliance with Follow-Up Visit*:**

Ensuring that families attend the 5-year follow-up visit within the designated time frame (+ 3 months) may present logistical challenges. To mitigate this, parents were contacted well in advance and provided with flexible scheduling options. For families unable to visit the study centre, alternative methods (such as phone interviews) were offered.

***Continuity of Care*:**

As participants transition from the first assessment to the follow-up assessment, there is the potential for loss of contact or reduced engagement with the study. To maintain continuity, regular communication with families were prioritized when feasible, ensuring that they remain informed about the importance of the follow-up visit and the benefits of continued participation in the study.

***Longitudinal Data Analysis*:**

Analysing data from both assessments presents a challenge in terms of integrating short-term and long-term outcomes. The statistical analysis will need to account for the different types of data (e.g., bi-monthly assessments during the first 6 months of life versus retrospective data at 5-year follow-up) and ensure that any confounding factors are adequately controlled.

By addressing these challenges through careful planning and participant support, the study aims to provide a clear and comprehensive understanding of the long-term effects of early nutritional interventions on childhood allergies and growth.

9 4. **Conclusion**

The A.R.T. 5-year follow-up study provides critical long-term data on the impact of early nutritional interventions on allergy prevention in high-risk infants. While breastfeeding remains the gold standard, findings suggest that pHF may offer some protective benefits against allergic diseases without compromising growth. However, the variability in outcomes across studies underscores the need for product-specific evaluations.
This study reinforces the importance of long-term follow-up in allergy research to refine infant feeding recommendations. Future research should focus on extending follow-up beyond early childhood. The findings contribute to the evolving evidence base, helping to inform clinical practice and public health guidelines on early-life nutrition and allergy prevention.

Protocol references

[1] B. Taylor, “The natural history of food allergy in infancy and childhood,” *Clin Rev Allergy*, vol. 4, pp. 53–104, 1986.

[2] Y. Katz, M. R. Goldberg, N. Rajuan, A. Cohen, and M. Leshno, “The prevalence and natural course of food protein–induced enterocolitis syndrome to cow’s milk: A large-scale, prospective population-based study,” *Journal of Allergy and Clinical Immunology*, vol. 127, no. 3, pp. 647-653.e3, Nov. 2011, doi: 10.1016/j.jaci.2010.12.1105.

[3] A. Høst, “Frequency of cow’s milk allergy in childhood,” *Annals of Allergy, Asthma & Immunology*, vol. 89, no. 6, pp. 33–37, Nov. 2002, doi: 10.1016/S1081-1206(10)62120-5.

[4] H. Wen *et al.*, “Prediction of atopic dermatitis in 2-yr-old children by cord blood IgE, genetic polymorphisms in cytokine genes, and maternal mentality during pregnancy,” *Pediatric Allergy and Immunology*, vol. 22, no. 7, pp. 695–703, Nov. 2011, doi: 10.1111/j.1399-3038.2011.01177.x.

[5] C. Caffarelli *et al.*, “Cow’s Milk Allergy in Breastfed Infants: What We Need to Know About Mechanisms, Management, and Maternal Role,” *Nutrients*, vol. 17, no. 11, p. 1787, Nov. 2025, doi: 10.3390/nu17111787.

[6] T. Boutsikou *et al.*, “The Impact of Infant Feeding Regimen on Cow’s Milk Protein Allergy, Atopic Dermatitis and Growth in High-Risk Infants during the First 6 Months of Life: The Allergy Reduction Trial,” *Nutrients*, vol. 15, no. 11, Jun. 2023, doi: 10.3390/nu15112622.

[7] D. A. Osborn, J. K. H. Sinn, and L. J. Jones, “Infant formulas containing hydrolysed protein for prevention of allergic disease,” *Cochrane Database of Systematic Reviews*, no. 10, 2018, doi: 10.1002/14651858.CD003664.pub6.

[8] C. M. F. Kneepkens and P. L. P. Brand, “Clinical practice: Breastfeeding and the prevention of allergy,” Aug. 2010. doi: 10.1007/s00431-010-1141-7.

[9] H. A. Sampson *et al.*, “Food allergy: A practice parameter update—2014,” *Journal of Allergy and Clinical Immunology*, vol. 134, no. 5, pp. 1016-1025.e43, Nov. 2014, doi: 10.1016/j.jaci.2014.05.013.

[10] A. W. Burks *et al.*, “NIAID-Sponsored 2010 Guidelines for Managing Food Allergy: Applications in the Pediatric Population,” *Pediatrics*, vol. 128, no. 5, pp. 955–965, Nov. 2011, doi: 10.1542/peds.2011-0539.

[11] L. T. Yokanovich, R. D. Newberry, and K. A. Knoop, “Regulation of oral antigen delivery early in life: Implications for oral tolerance and food allergy,” *Clinical & Experimental Allergy*, vol. 51, no. 4, pp. 518–526, Nov. 2021, doi: 10.1111/cea.13823.
[12] “Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel,” *Journal of Allergy and Clinical Immunology*, vol. 126, no. 6, pp. S1–S58, Nov. 2010, doi: 10.1016/j.jaci.2010.10.007.
[13] M. Sekkidou, E. Philippou, P. K. Yiallouros, and N. Nicolaou, “Is it time to reconsider the role of partially hydrolyzed infant formulas in the prevention of allergic diseases?,” *Allergol Immunopathol (Madr)*, vol. 53, no. 3, pp. 181–193, 2025, doi: 10.15586/aei.v53i3.1317.
[14] A. von Berg *et al.*, “The effect of hydrolyzed cow’s milk formula for allergy prevention in the first year of life: The German Infant Nutritional Intervention Study, a randomized double-blind trial,” *Journal of Allergy and Clinical Immunology*, vol. 111, no. 3, pp. 533–540, Nov. 2003, doi: 10.1067/mai.2003.101.
[15] T. Hays and R. Wood, “A Systematic Review of the Role of Hydrolyzed Infant Formulas in Allergy Prevention,” *JAMA Pediatr*, vol. 159, no. 9, pp. 810–816, 2005, doi: 10.1001/archpedi.159.9.810.
[16] R. J. Boyle *et al.*, “Hydrolysed formula and risk of allergic or autoimmune disease: systematic review and meta-analysis,” *BMJ*, p. i974, Nov. 2016, doi: 10.1136/bmj.i974.
[17] X. Li *et al.*, “Infant Formulas With Partially or Extensively Hydrolyzed Milk Proteins for the Prevention of Allergic Diseases: A Systematic Review and Meta-Analysis of Clinical Trials,” *Advances in Nutrition*, vol. 15, no. 5, p. 100217, May 2024, doi: 10.1016/J.ADVNUT.2024.100217.
[18] A.-W. Chan *et al.*, “SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials,” *BMJ*, vol. 346, no. jan08 15, pp. e7586–e7586, Nov. 2013, doi: 10.1136/bmj.e7586.
[19] N. Nicolaou *et al.*, “The Risk Reduction Effect of a Nutritional Intervention With a Partially Hydrolyzed Whey-Based Formula on Cow’s Milk Protein Allergy and Atopic Dermatitis in High-Risk Infants Within the First 6 Months of Life: The Allergy Reduction Trial (A.R.T.), a Multicenter Double-Blinded Randomized Controlled Study,” *Front Nutr*, vol. 9, Nov. 2022, doi: 10.3389/fnut.2022.863599.
[20] A. Minasyan, A. Babajanyan, D. E. Campbell, and R. Nanan, “Validation of a Comprehensive Early Childhood Allergy Questionnaire,” *Pediatric Allergy and Immunology*, vol. 26, no. 6, pp. 522–529, Nov. 2015, doi: 10.1111/pai.12415.

[21] T. Boutsikou *et al.*, "The Impact of Infant Feeding Regimen on Cow's Milk Protein Allergy, Atopic Dermatitis and Growth in High-Risk Infants during the First 6 Months of Life: The Allergy Reduction Trial," *Nutrients*, vol. 15, no. 11, p. 2622, Nov. 2023, doi: 10.3390/nu15112622.
[22] R. Pancheva *et al.*, "Early life acute infections and risk for cow's milk protein allergy or atopic dermatitis at 6 months of age in high risk for allergy infants," *Front Pediatr*, vol. 12, Nov. 2024, doi: 10.3389/fped.2024.1424331.
[23] M. I. Asher *et al.*, "International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods," *European Respiratory Journal*, vol. 8, no. 3, pp. 483–491, Nov. 1995, doi: 10.1183/09031936.95.08030483.
[24] L. Libuda *et al.*, "Full Breastfeeding and Allergic Diseases—Long-Term Protection or Rebound Effects?," *Nutrients*, vol. 15, no. 12, p. 2780, Nov. 2023, doi: 10.3390/nu15122780.
[25] I. Stewart and C. I. Bloom, "Considerations for conducting and interpreting long-term follow-up of intervention studies: avoiding spoiled milk," *Thorax*, vol. 76, no. 11, pp. 1067–1068, Nov. 2021, doi: 10.1136/thoraxjnl-2021-217352.
[26] R. L. Peters and K. P. Perrett, "Monitoring changes in infant feeding practices after changes to guidelines for food allergy prevention," *Medical Journal of Australia*, vol. 212, no. 6, pp. 256–257, Nov. 2020, doi: 10.5694/mja2.50535.
[27] J. G. Mathias, H. Zhang, N. Soto-Ramirez, and W. Karmaus, "The association of infant feeding patterns with food allergy symptoms and food allergy in early childhood," *Int Breastfeed J*, vol. 14, no. 1, p. 43, Nov. 2019, doi: 10.1186/s13006-019-0241-x.
[28] A. von Berg *et al.*, "Allergies in high-risk schoolchildren after early intervention with cow's milk protein hydrolysates: 10-year results from the German Infant Nutritional Intervention (GINI) study," *Journal of Allergy and Clinical Immunology*, vol. 131, no. 6, pp. 1565-1573.e5, Nov. 2013, doi: 10.1016/j.jaci.2013.01.006.
[29] A. von Berg *et al.*, "Allergic manifestation 15 years after early intervention with hydrolyzed formulas – the <scp>GINI</scp> Study," *Allergy*, vol. 71, no. 2, pp. 210–219, Nov. 2016, doi: 10.1111/all.12790.
[30] P. Rzehak *et al.*, "Long-term effects of hydrolyzed protein infant formulas on growth—extended follow-up to 10 y of age: results from the German Infant Nutritional Intervention (GINI) study," *Am J Clin Nutr*, vol. 94, pp. S1803–S1807, Nov. 2011, doi: 10.3945/ajcn.110.000679.

Acknowledgements

This research was funded by FrieslandCampina Nederland, which provided the infant formulas and financial support to the participating centers to conduct the study. **Funding:** The A.R.T. trial is funded by FrieslandCampina, the manufacturer of the pHF and SF used, in collaboration with the research institutions involved in the study. The funding covers all aspects of the trial, including data collection, participant follow-up. The sponsors have no role in the direct execution of the study, and the trial is conducted independently by the research team to maintain scientific integrity and minimize bias. Full transparency regarding the funding and any potential conflicts of interest is ensured through adherence to international clinical trial reporting standards. Following analysis, study results will be compiled into a report for FrieslandCampina and submitted for publication in an international peer-reviewed journal.