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Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis: Protocol of the Goat Infant Formula Feeding and Eczema (GIraFFE) study

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Abstract

Introduction Atopic dermatitis (AD) is a chronic, inflammatory skin condition significantly affecting quality of life. A small randomized trial showed an approximately 1/3 lower incidence of AD in goat milk formula-fed compared to cow milk formula-fed infants. However, due to limited statistical power AD incidence difference was not found significant. This study aims to explore a potential risk reduction of AD by feeding a formula based on whole goat milk (as a source of protein and fat) compared to a formula based on cow milk proteins and vegetable oils.

Methods and analysis This two-arm, parallel, randomized, double-blind, controlled nutritional trial shall enrol up to 2296 healthy term born infants up to 3 months of age if parents choose to start formula feeding. Ten study centres in Spain and Poland take part. Randomized infants receive investigational infant and follow-on formulas either based on whole goat milk or on cow milk until the age of 12 months. The goat milk formula has a whey:casein ratio of 20:80 and about 50% of the lipids are milk fat from whole goat milk, whereas the cow milk formula, used as control, has a whey:casein ratio of 60:40 and 100% of the lipids are from vegetable oils. The energy and nutrient levels in both goat and cow milk formulas are the same. The primary endpoint is the cumulative incidence of AD up to the age of 12 months diagnosed by study

personnel based on the UK Working Party Diagnostic Criteria. The secondary endpoints include reported AD diagnosis, measures of AD, blood and stool markers, child growth, sleep, nutrition and quality of life. Participating children are followed until the age of 5 years.

Ethics and dissemination Ethical approval was obtained from the ethical committees of all participating institutions.

Trial registration number NCT04599946 (registered on 23.10.2020)

Strengths and limitations of this study

- Potential confounding is minimized due to the randomized study design.
- A multicentre study design with sites in different countries increases external validity of study results.
- The follow-up until five years of age allows to examine long-term effects of infant feeding.
- Effect sizes may be limited due to the short-time period of consuming study formula as the only food.

Keywords

Goat milk; infant formula; clinical trial; atopic dermatitis; eczema; infant nutrition; food allergies.

Word Count: 3709

INTRODUCTION

Atopic dermatitis (AD), also known as eczema or atopic eczema, is a chronic, inflammatory, pruritic skin condition that frequently occurs in children [1] and adults. It is characterized by intense itch, recurrent eczematous lesions, and a fluctuating course. AD affects 15 to 30% of children in industrialized countries [2]. It is reported to often be the prelude to an atopic march including food allergies, asthma, and allergic rhino-conjunctivitis [3].

The clinical phenotype observed in individuals with AD is variable. To support diagnosis, several sets of criteria considering the intermittent nature of AD and possible fluctuations in AD activity, have been developed including the UK Working Party criteria [4-6]. Validated scoring systems such as the Scoring Atopic Dermatitis (SCORAD) or the Patient-Oriented Eczema Measure (POEM) have been introduced [7 8].

In a cohort of 620 Australian children at high risk for atopic diseases, a clear association between AD severity and the occurrence of IgE-mediated food allergies was shown [9]. This agrees with the findings in other studies and was extended to aeroallergens [10]. A potential explanation for this observation is the dual allergen exposure hypothesis (epicutaneous and intestinal), which posits that epicutaneous food sensitization occurs through the impaired skin barrier in AD, enabling allergen penetration and cytokine dysregulation, which leads to clinical food allergy [11]. Thus, food allergy may occur in a situation of impaired skin barrier integrity in combination with a failure to achieve oral tolerance. Food allergens induce flares in a considerable proportion of infants with moderate-to-severe AD [12].

Taking the considerable loss of quality of life and associated disease risks in children affected by AD into account, infant feeding schemes for the general population associated with a decreased risk of AD manifestation would be highly desirable. So far, no generally accepted strategies for primary prevention of AD are available. For infants at high risk of developing AD, a 4-month period of breastfeeding might be advisable, but results are controversial [13]. Formulas based on hydrolyzed proteins, as well as prebiotics and probiotics, were reported to provide protective effects but results are inconsistent [14 15 16].

Studies in mice and guinea pigs have suggested that goat milk is less allergenic than cow milk [17-19] although such differences are not confirmed in all studies [20]. The allergenic protein α_{s1}-casein is the dominant casein in cow milk, with 12–15 g/L. In contrast, goat milk has variable levels of this protein dependent on genotype, ranging from 0.9 to 7 g/L [21]. During digestion, caseins from goat milk are broken down to a greater extent than those from cow milk, corresponding to a potentially lower allergenic burden from goat milk [21].

A multicenter, double-blind, controlled feeding trial in Australia found that an infant formula based on cow milk proteins and vegetable oils and a formula based on whole goat milk were both well tolerated and supported physiological growth [22]. This is in agreement with two other studies performed in New Zealand [23] and China [24], which tested formulas based on whole goat milk and goat milk protein, respectively. The Australian study also showed an approximately 1/3 lower incidence of AD in the whole goat milk formula-fed compared to the standard cow milk formula-fed infants [22], but the difference was not statistically significant given the trial was not powered to detect differences in AD incidence. Of interest, in a murine model of AD inclusion of goat milk lipids into the diet reduced inflammation [25]. The complex variety of lipids

provided in whole milk fat, including the polar lipid species of milk fat globule membranes, might be beneficial for gut and skin barrier functions [26].

We hypothesize that goat and cow milk-based infant formulas could differently affect blood-based biomarkers, the gut microbiome and the risk of AD development. Therefore, the Goat Infant Formula Feeding and Eczema (GIraFFE) study tests whether infant feeding with a formula based on whole goat milk (protein and fat) reduces the risk of developing AD when compared to a formula based on cow milk proteins and vegetable oils. Secondarily, the study aims to contribute to the identification of risk factors for AD, elucidation of the mechanistic understanding of the immune system development and to provide a resource for studying other questions related to infant nutrition and development.

Primary Objective

The primary objective of this trial is to determine the relative risk of developing AD in the first 12 months in infants fed a formula based on whole goat milk compared to infants fed a formula based on cow milk.

Secondary Objectives

The secondary objectives are related to AD and other atopic diseases but also to the child´s growth and wellbeing, including infant metabolism and gut health, in the first 5 years of life. All outcomes will be compared for an effect of the study formula treatment (goat formula vs cow formula). The study will also explore associations of AD and other atopic diseases and overall development, and aims to identify risk indicators.

METHODS AND ANALYSIS

Study design and population

The GIraFFE study is a randomized, double-blind, parallel-group clinical trial to study the effect of feeding infant a whole goat milk or a whey-adjusted cow milk formula during the first year of life on the risk of allergy and other health outcomes, including growth and quality of life, in the first 5 years of life. The study is led by the key principal investigator Professor Dr. Berthold Koletzko and conducted as a multicentre trial in currently 4 study centres in Poland and 6 study centres in Spain, which all have local principal investigators.

The study population consists of healthy term infants of parents who decided to start formula feeding, without a preselection for children with an increased risk for AD. The study teams proactively promote, support and protect breastfeeding. Only infants of parents who decided to start formula feeding are enrolled into the study but are encouraged to continue partial breastfeeding after enrolment. The infants participating need to fulfil the criteria depicted in Table 1.

Table 1: Inclusion and Exclusion criteria of the GIraFFE study

Inclusion criteria	Exclusion criteria
• Written informed consent (signed and dated) of the child's parent(s)/caregiver(s), indicating that the child's parent(s)/caregiver(s) has/have been informed of all pertinent aspects of the study • Infant was born full term (≥37 weeks +0 days and ≤ 41 weeks +6 days of gestation) • Age at enrolment < 90 days • Infant birth weight ≥2.5 kg and ≤4.5 kg • Infant is born from a singleton pregnancy • Child's parent(s)/caregiver(s) is/are of legal age of consent • The child's parent(s)/caregiver(s) have sufficient local language skills to understand the study information, the informed consent, and to comply with the study procedure • The child's parent(s)/caregiver(s) is/are willing and deemed able to	• Diagnosed disorder considered to interfere with nutrition, growth, or development of the immune system • Participation of the child in any other interventional trial or participation of the mother in any intervention trial with child follow-up • Infant has a doctor's diagnosis of AD or a severe widespread skin condition prior to randomization that makes the detection or assessment of AD difficult • Infant has regularly (on average at least 3-4 days a week, at least one bottle per day) consumed an infant formula other than study formula for more than 4 weeks prior to enrolment • Cow's milk allergy or intolerance of the child • Institutionalized infant

	fulfil the requirements of the study protocol and procedures • Mother has expressed the intention to partially (in combination with breastfeeding) or fully formula-feed

Study formulas

Participants are randomly assigned to receive one of the two formulas manufactured by Dairy Goat Co-operative (N.Z.) Ltd (Hamilton, New Zealand). The goat milk formula is already marketed as Capricare; it is based on whole goat milk as a source of protein (20:80 whey:casein ratio) and goat milk fat contributes 50% of total fat. The control formula is based on cow skim milk and whey protein powders (60:40 whey:casein ratio) and vegetable oils as the almost only source of fat. The study formulas are isocaloric, have the same macronutrient composition and are provided as infant and follow-on formulas (Supplementary Table 1). The composition of all formulas complies with European Commission Delegated Regulation (EU) 2016/127.

The key differences are 1) the source of milk from cows or goats, 2) the whey:casein ratio, and 3) the fat source.

Study product intake and compliance

Feeding of study formulas can begin immediately after enrolment, but must start no later than the age of 4 months and continues until the age of 12 months. The study formula is fed ad libitum and shall be the only formula given to the participating infant. If infants do not consume at least some study formula before the infant is 4 months old, the infants are excluded from the study.

Preparation and feeding guidelines are identical for both study formulas and are in agreement with common practice. The study teams advise not to use follow-on formula prior to the infant age of 6 months, but it is the parent's decision whether and when to introduce follow-on formula. Compliance is defined as a continuous study formula consumption over the whole intervention period without any breaks longer than 3 consecutive days and no introduction of solid foods before the age of 4 months. Compliance will be checked at all scheduled study

contacts and plausibility of continuous consumption will be checked by the number of consumed cans.

Outcome measurements

The primary endpoint of the GIraFFE study is the cumulative incidence of AD up to the age of 12 months diagnosed by study personnel, defined as meeting the UK Working Party Diagnostic Criteria for AD. The secondary endpoints are listed in Table 2.

Table 2: Secondary endpoints

Secondary endpoints
• Cumulative incidence of study personnel-diagnosed AD, defined as meeting the UK Working Party Diagnostic Criteria for AD, up to the age of 24 and 60 months, as long-term follow-up of the primary outcome.
• Cumulative incidence of parental reported diagnosis of AD up to 12, 24 and 60 months, defined as meeting the UK Working Party Diagnostic Criteria for AD, in a telephone interview or parental report of a non-study doctor diagnosis in addition to the study diagnosis of AD.
• Point incidence of study diagnosed and parental reported AD, defined as meeting the UK Working Party Diagnostic Criteria for AD at 4, 6, 12, 24 and 60 months.
• Age at first study diagnosis, parental report-based study diagnosis or parental report of a diagnosis of AD by a non-study doctor up to 12, 24 and 60 months.
• AD severity in children with diagnosed (study diagnosis or reported diagnosis) AD, using SCORAD questionnaire completed by study personnel at all face-to-face visits (4, 6, 12, 24 and 60 months) and POEM questionnaire completed by parents at all scheduled contacts (4, 6, 8, 10, 12, 18, 24, 36, 48 and 60 months).
• Cumulative use of eczema-related medication or skin care up to 12, 24 and 60 months.
• Parental report of a clinical diagnosis of food allergy (12, 24 and 60 months).
• Parental reported hay fever and asthma-related diseases up to 12, 24 and 60 months.
• Anthropometric measures (weight-for-age, length-for-age and BMI-for-age z-scores) at baseline, 4, 6, 12, 24 and 60 months.

- Parental report of gastrointestinal symptoms (Infant Gastrointestinal Symptom Questionnaire, IGSQ) and sleep (Brief Infant Sleep Questionnaire, BISQ) during the intervention period (4, 6 and 12 months).
- Quality of life in children using the Infant Toddler Quality Of Life questionnaire (ITQOL) filled by parents at 4, 12, 24 and 60 months.
- Nutrition during the intervention period (4, 6, 8, 10, 12 and 60 months).
- Biochemical measures:
    - Allergic sensitization (total and specific IgEs including cow milk protein and goat milk) at 12 and 60 months
    - Blood lipids, metabolome, lipidome and further exploratory markers at 4, 12 and 60 months
    - Gut microbiome at 4, 12 and 60 months

Sample size

The number of subjects to be studied was based on the incidence of AD in the population and the effect size to be detected. Reported AD incidence estimates for young children in Spain and Poland are 13% and 17%, respectively [27 28]. The previous study comparing goat and cow milk formulas had indicated a risk reduction for AD incidence of 30% [22]. Thus; we assume a cumulative incidence of AD at 15% in the first 12 months of life, based on the cited data, and a 30% clinically relevant risk reduction by whole goat milk formula. A sample size of 861 infants per group is required to set the significance level to 0.05 and statistical power to 80%. We estimate the dropout rate until the age of 12 months to be 25%. Thus, 1148 infants per group (in total 2296) need to be studied. If the dropout rate turns out to differ markedly from the assumption, the number of infants to be recruited may be adjusted during the study.

Recruitment

Precautions are taken to ensure that recruitment does not undermine breastfeeding intentions and practice. Due to differences in health care systems and local infrastructure the way to approach and recruit subjects is different for each study centre. In most cases those families who expressed their decision to partially or fully formula-feed are made aware of the study in paediatric practices or primary health care centres. In any case, parents are not informed about

the study until they had decided to feed the baby with formula or both formula and breastmilk, in order not to interfere with breastfeeding. The recruitment of study participants has started in January 2021 and is currently ongoing in all ten study centres.

Blinding and randomized allocation of study formulas

The study is double blinded using four different 3-character codes, two for each study product. Study personnel, biostatistician, data manager, trial monitor, laboratory analysts, and all persons involved in the organization and conduct of the study and study participants are blinded. Study products are shipped to the participating families and the sites by logistic partners.

For the allocation of the subjects to the four study codes minimisation randomization is applied with centres as the only strata [29 30]. The dynamic randomization method minimizes imbalances in age at randomization and sex. A random element makes assignment unpredictable with a maximal group difference of +/- 6 children allowed.

DATA COLLECTION, MANAGEMENT AND ANALYSIS

Data collection and management

Figure 1: Schematic representation of the study design

During the intervention, study centre visits are planned at enrolment (= baseline and randomization), at 4, 6 and 12 months of age, and during the follow-up visits at 24 and 60 months (Figure 1). Telephone contacts after enrolment and at 2 and 3 months of age are done, depending on the age at enrolment. After the face-to-face visits at age 4 and 6 months, which aim to collect data during the phase of dominating formula feeding (4 months) and the age of high incidence of AD in the Australian study at age 6 months [22], phone calls are scheduled at age 8 and 10 months for further data collection and to support protocol compliance and study logistics during the intervention period. During the follow-up, telephone calls are performed at 18, 36, 48 months of age to collect data and enhance contacts with the participating families.

An initial screening for eligibility is performed at the first contact with potentially participating families, and at the enrolment visit prior to randomization the subject´s suitability according to inclusion and exclusion criteria is confirmed. Families willing to participate sign the informed consent form. A template informed consent form is enclosed in the supplemental material. At the baseline visit information about atopic diseases of parents and siblings, pregnancy information, birth data, socio-economic background, the home environment, the child´s medical history and details of feeding practices since birth are collected. At all study visits anthropometric measurements are performed.

The UK Working Party Diagnostic Criteria are used for AD diagnosis at enrolment and at all subsequent visits and telephone calls until the age of 60 months. Criteria are adapted for children under the age of 12 months in respect to time frame and body areas considered and at the telephone calls, when no direct visual inspection is possible and parental report at the visit day are documented.

Two questionnaires are used for the assessment of the severity of diagnosed AD (study diagnosis or reported diagnosis): 1) SCORAD questionnaire at all face-to-face visits 2) POEM questionnaire at all examination time points up to 60 months of age. For all children who were ever study-diagnosed with AD, this reflects the objective view of trained medical personnel (SCORAD) and the more subjective view of the parents (POEM).

The introduction of complementary feeding, use of cow milk and cow milk products, allergenic foods, use of beverages and food preferences is assessed with questionnaires at 4, 6, 8, 10 and 12 months. At the 12-month and 60-month visits, Food Frequency Questionnaires (FFQ) are

used for a more detailed assessment of dietary habits. The FFQ was modified according to the age of children, based on FFQ version of the Identification and prevention of dietary- and lifestyle-induced health effects in children and infants project[31].

During all telephone calls and visits in the intervention period, intake and acceptance of the formula is assessed as compliance indicator. For adverse event recording, participating families are asked in all scheduled visits and telephone calls for hospitalizations, illness and any medication of the child.

Parents are explicitly asked for a doctor's diagnosis of food allergies at 12, 24 and 60 months with a specific focus on cow milk, egg, peanuts, soy, and fish. Furthermore, asthma, bronchitis/bronchiolitis, wheezing and allergic rhinitis at the 60-month visit with distinction between self-observation and doctor-diagnosis are assessed.

During the intervention period, questionnaires about sleep (BISQ) and gastrointestinal problems (IGSQ) are applied at the face-to-face visits. Furthermore, the Infant Toddler Quality Of Life questionnaire (ITQOL) is completed at 4, 12, 24 and 60 months by parents. Parents are asked at enrolment, and at all contacts from 4 months on about general skin care and if there has been a prescription of topical treatment like corticosteroid or other immunosuppressive therapies by a physician since last visit.

Data are collected primarily with a web-based online database developed by CSAM MedSciNet U.K. Ltd with direct data entry by study personnel and participating families as default option. The use of paper forms is limited to situations where the direct input into the database is technically not possible or not wished by parents. Furthermore, copies of signed consent forms are stored electronically. All procedures are checked for general data protection regulation conformity by a LMU data protection officer.

Biosamples

Blood collection is planned at the 4, 12 and 60-month visits. Standard operating procedures are in place. Highest priority is given to the analysis of atopy-related parameters such as total IgE, specific IgEs for cow and goat milk protein, as well as further frequent allergens, and inflammation markers. Serum lipids (total cholesterol, HDL-cholesterol, triglycerides) and lipidomic and metabolomic analyses aim at describing the metabolism of the infants in respect to formula consumed and for the identification of biochemical risk markers or eventual

metabolic consequences of AD. As a safety indicator, full blood count is taken from all blood samples. If corresponding consent has been obtained, Filaggrin genotype will be determined and further genetic analysis performed if additional funding is granted.

For microbiome analyses, stool samples are collected at 4, 12, and 60 months in a subgroup of 600 infants. According to the standardized procedures, samples should be frozen at -20°C within less than 15 min after collection; samples have to be transferred to a -80°C freezer within a week.

Adverse Events

Adverse events (AEs) are recorded according to a standardised protocol including an opinion on the assumed relation to the intervention and a categorization of the AEs. During the intervention and until 30 days after study product intake all safety events fulfilling the following criteria are reported as AEs.

- Child was treated with:
  - Medication > 14 days
  - Oral antibiotics
  - Inhalation therapy
  - Steroids, salbutamol, antihistamines, montelukast
- Child was hospitalized
- Child was treated with a special diet > 7days
- Child interrupted the intake of the study product > 1 day or completely discontinued consumption

From 31 days after the last product intake only safety events, fulfilling the applicable criteria, and considered as potentially related to the intervention or that may influence study outcomes are reported as AE.

Any AE that results in death, is life-threatening, requires hospitalization or results in persistent or significant disabilities is classified as serious adverse event (SAE). The Principal Investigators of the individual study centres review all SAEs at their centre and provide an opinion, including a comment on the relation to the intervention.

A clinical trial insurance has been set up.

Monitoring

An external monitor performed the study monitoring during the first ten months of the study recruitment. After this period monitoring activities are taken over by LMU researchers. Monitoring should improve the quality of the collected data but mainly focuses on the compliance of all local study procedures with the protocol, established SOPs and good clinical practice. Besides on-site monitoring, additional remote monitoring is also performed.

A Data and Safety Monitoring Board has been established, whose primary responsibilities are to review and evaluate data for participant safety and study progress. Based on the accumulated study data, the board makes recommendations concerning continuation, modification, or eventual termination of the GIraFFE study.

Statistical analysis

Statistical analyses are scheduled when all recruited infants have passed V12, V24 and V60, respectively. All primary and secondary analyses including methods to deal with missing data and sub-group analyses are to be specified in a Statistical Analysis Plan, which is finalized prior to database lock and unblinding.

As primary statistical analysis, a comparison of the cumulative incidence of children with AD until 12 months of age between the goat milk formula group and cow milk formula group is planned. For this analysis, a Generalized Estimating Equation Poisson model with a log link and robust standard errors by sandwich estimators of variance will be used [32].

The findings are compared with further adjusted models that include major influencing factors of AD frequency, including country, sex, Filaggrin genotype, parental atopic diseases, parental AD, antibiotic usage, family size and socio-economic status. Furthermore, interactions of Filaggrin mutations, the number of immediate family members with AD or other atopic disease with AD frequency shall be investigated. If effect modification by one of the mentioned predictive covariates is significant at the 5% level, subgroup analyses for each category will be presented.

Secondary analyses will look at the secondary objectives with similar statistical approaches.

Ethics and Dissemination

Ethical approval was obtained from the ethical committee of the LMU University Hospital Munich, Germany (Nr. 20-188; ethikkommission@med.uni-muenchen.de) and the ethical committees of all ten study centres, Hospital Universitario La Paz, Madrid (Ref. 47/322688.9/20; Ref: 47/748801.9/21); CEIC Aragón (CEICA), Zaragoza (C.P. - C.I. PI20/098); Hospital Clínico Universitario de Valencia Ref. CEIm 2020/219); Institut d'Investigació Sanitària Pere Virgili, Reus/Tarragona (Ref. CEIM: 057/2020); CEIM/CEI Andalucía, Delegación Provincial de Granada (Ref. CEIM/CEI: 1134-M1-20); Hospital Universitario Torrecárdenas, Almeria (Ref. CEIM: 109/2019), Warmi ń sko – Mazurskiej Izbie Lekarskiej w Olsztynie (Nr 1/2020/VII); Poznań University of Medical Sciences (No 436/20); University of Rzeszów (No. Number 05/07/2020); Instytucie "Pomnik-Centrum Zdrowia Dziecka" (12/KBE/2020).

Currently protocol version 1.1 is valid since 25.06.2020. The ethical committees will approve all protocol amendements prior to implementation.

Patient and Public Involvement

The protocol for the study including all procedures related to subject safety and protection of personal data was predominantly developed at a public hospital, but without specific patient consultations.

Public dissemination and data availability

Researchers and sponsor are committed to publish the study findings in peer-reviewed international scientific journals. Dissemination of study results may also include posting of a synopsis online, abstracts submitted to and presentations at scientific conferences, and other dissemination activities including social media.

After a delay period for full scientific evaluation, the remaining biosamples and associated data of participants, for whom respective consent is available, will be transferred into a registered biobank (Hauner biobank, LMU München). Data and samples will be accessible for other researchers according to the biobank regulations.

Funding, role of the sponsor and investigators

The study products are manufactured and provided to participants by the study sponsor (Dairy Goat Cooperative (N.Z.) Ltd, Hamilton, New Zealand). The sponsor has allocated a fixed budget

to each of the institutions hosting the study centre and the key principal investigator with his team to conduct the study.

The sponsor, the site principal investigators and the key principal investigator have agreed and fixed in the study protocol that the final decision making power on the study rests with the trial steering committee, which includes the key principal investigator, all site principal investigators, and the sponsor. The trial steering committee also takes decisions on further grant applications to fund additional analyses of data and biosamples generated in GIraFFE.

Authors´ Statement

JF produced the first draft of the manuscript and all co-authors critically reviewed the manuscript and approved the final version. The members of the GIraFFE study group contributed to the realization of the study.

The GIraFFE study group consists of the following members: Carme Rubio-Torrents, Ester Parada-Ricart, Natalia Ferré, Veronica Luque (Tarragona/Reus); Encarnación López-Ruzafa, Melinda Moriczi (Almeria); Elena Crehuá-Gaudiza, Cecilia Martínez-Costa (Valencia); Gerardo Rodriguez, María Luisa Álvarez, Cristina Guillén, María Perán, Laura García, Sheila García (Zaragoza); Bibiana Chinea, Ariadna Witte, Esperanza Escribano (Madrid); Jose Antonio García-Santos, Mireia Escudero-Marín, Rocío Bonillo-León (Granada); Janusz Książyk, Alicja Syc, Aleksandra Żyła-Pawlak (Warsaw); Artur Mazur (Rzeszów); Małgorzata Jamka, Aleksandra Lisowska (Poznań).

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Conflict of interest

SG is an employee of DGC (Dairy Goat Cooperative (N.Z.) Ltd, Hamilton, New Zealand).

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Colin Prosser (DGC), Philipp Schwarzfischer and Sandra Unterschemmann (LMU) contributed to the development or the implementation of the protocol.

Supplemental Material

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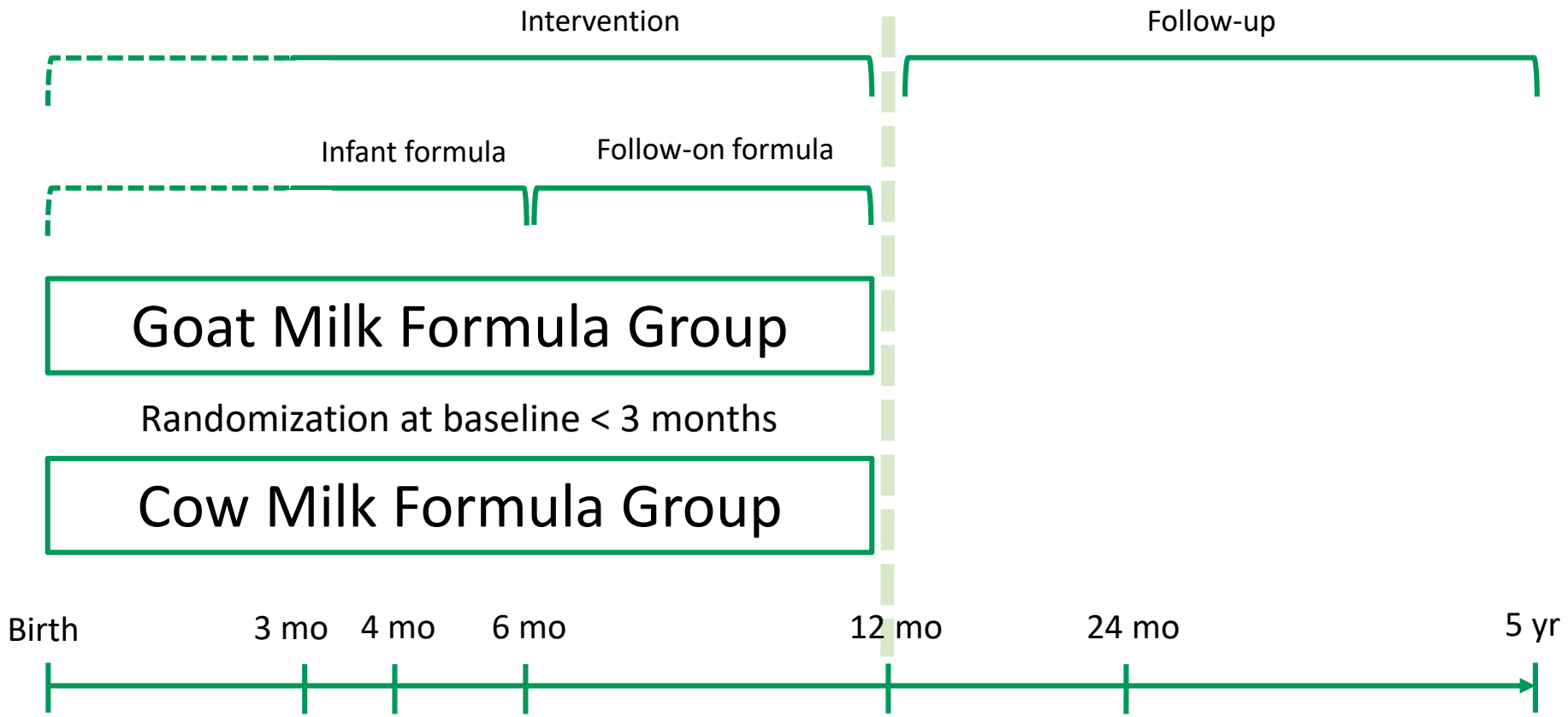

Intervention
Follow-up
Infant formula
Follow-on formula
Goat Milk Formula Group
Randomization at baseline < 3 months
Cow Milk Formula Group
Birth
3 mo
4 mo
6 mo
12 mo
24 mo
5 yr

Supplemental Material

Supplementary Table 1: Composition of the study infant and follow-on formulas:

Nutrients	Unit	**Whole goat infant formula** per 100 mL of prepared feed*	**Cow infant formula** per 100 mL of prepared feed*	**Whole goat follow-on formula** per 100 mL of prepared feed*	**Cow follow-on formula** per 100 mL of prepared feed*
Energy	kj	270	270	270	270
	kcal	65	65	65	65
Fat	g	3.4	3.4	3.4	3.4
Milk fat	% total fat	46	2	46	2
Saturated fatty acids	g	1.3	1.3	1.3	1.3
Docosahexaenoic acid (DHA)	mg	15.5	15.5	15.5	15.5
Arachidonic acid (ARA)	mg	16.1	16.1	16.1	16.1
Carbohydrate	g	7.3	7.3	7.3	7.3
Sugars (lactose)	g	7.1	7.1	7.1	7.1
Protein	g	1.3	1.3	1.3	1.3
Whey	% total protein	20	60	20	60
Casein	% total protein	80	40	80	40

* Prepared as follows: 4.3 g of powder + 30 mL of water (12.9 g of powder per 100 mL of milk).

[1]Retinol equivalents, [2]alpha-tocopherol equivalents, [3]dietary folate equivalents

Parent information and consent

Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis (GIraFFE Study)

Study registration:

NCT04599946 at clinicaltrials.gov

Please read this information carefully. The study staff will answer any questions you may have.

The GIraFFE study was examined by the Ethics Committee and the Data Protection Officer of [your institution's approving committee/person] *and obtained a favourable opinion.*

You will receive a copy of this letter for your records.

PREAMBLE: *Exclusive breastfeeding is the ideal and healthiest way to feed your infant. This study is only offered to families whose children are completely formula-fed or whose parents have decided to provide mixed feeding (breastfeeding combined with formula). If your infant is receiving mixed feeding, but you would like to achieve exclusive breastfeeding, we can offer you support to achieve this, instead of participating in this study.*

Dear Family,

Thank you for your interest in our study that we are conducting together with partners in Spain, Poland and Germany. We want to find out if nutrition in early life affects the onset of atopic dermatitis (also known as eczema and atopic eczema). The study is called the GIraFFE study (**G**oat **I**nfant **F**ormula **F**eeding and **E**czema).

Why are we performing the study?

The increasing number of children with atopic dermatitis and allergies is a major medical problem. We are interested in understanding why some children develop allergies. Atopic dermatitis affects all age groups, but can be a particular concern for infants and small children. In a small study in Australia, infants were fed either a goat milk-based formula or a cow milk-based formula. A difference in the number of infants developing atopic dermatitis in first year of life was found. While goat milk and cow milk formula are both suitable for infant feeding if breastfeeding is not possible, they slightly differ in their composition, types of fat and proteins. These differences in composition might play a role in the development of atopic dermatitis or allergies. Understanding the role of these factors in the development of atopic dermatitis and allergies will help to choose the most suitable formula, and to improve formula composition and guidelines for infant nutrition.

Purpose of study

The aim of the GIraFFE study is to compare if formulas based on goat milk and cow milk have different effects on the development of atopic dermatitis and other related allergic diseases in a larger number of babies. The study formulas have the same composition of the essential nutritional components to support normal growth and

development of infants. Furthermore, we will assess and analyse stool bacteria and bio samples in the participating child as well as exploring other indicators of general health, development and metabolism.

Course of the study (see also Figure 1)

If you agree to participate, your baby will randomly be assigned to receive the cow or the goat milk formula. This randomization is important to exclude that any other factors related to food choice might cause a difference in eczema occurrence. To prevent any potential influence on the study results, neither you nor the study personnel will know which of the formulas your baby receives during the study. You will receive formula free of charge from enrolment until the study visit scheduled around your child's first birthday (age 12 months). After your child's first birthday, the formula supply will end and you will be free to choose what to feed your child, but the study itself will continue with following your child until the 5th birthday (age 60 months). A total of 2296 infants will participate in the GIraFFE study (distributed over 10 study centres in Poland and Spain). The overall study coordinator is Prof. B. Koletzko at the Dr. von Hauner Children's Hospital at the University of Munich, Germany. The local coordination will be done by XXX.

Participation in the GIraFFE study begins during the first three months of life. After the enrolment examination, further appointments for your child are planned here at [your institution] at the age of 4, 6, 12, 24 and 60 months. Every time you visit us, we will examine your child for signs of atopic dermatitis and measure height and weight. We will ask you questions about your child's health and general behaviour. In order to find out about the environment your child grows up in, we will initially ask you about your origin, education and family structure as well as cases of atopic dermatitis and allergic diseases in the family. In order to understand what your child eats and drinks apart from the study formula, we will ask you at each appointment which complementary foods you have already introduced to your child. At the age of 12 months, we also ask a little more about your child's dietary habits. During the first year of life, we also ask about the sleeping habits (BISQ questionnaire) and gastrointestinal comfort (IGSQ questionnaire) of your child. To assess the quality of life of your child, we will ask you to fill a slightly longer questionnaire (ITQOL questionnaire) at 4, 12, 24 and 60 months of age. Most of the questionnaires will be available online, so will be able to fill them online at home and reduce the time needed for each study visit.

If your child shows signs of atopic dermatitis, we record the severity with a standardized tool called SCORAD, when your child comes in for the next scheduled appointment. In addition, the POEM questionnaire is used to record the influence of atopic dermatitis on your child's quality of life.

In case you agree, we would like to take a small volume of blood (approx. 4-6 ml) from your child by a doctor or trained nurse during visits at 4, 12 and 60 months. We will be happy to share some of the results relevant for the assessment of your child's health such as blood count with you and your paediatrician. For blood sampling we offer applying local anaesthetic cream to the puncture site to avoid inconvenience for your child. At the same time points, we also ask you to collect some stool of your child. A kit for stool collection and instructions will be provided to you.

We will also contact you by phone shortly after enrolment and at 8 and 10 months to ask about the general health of your child, intake of the study formula, to check for signs of atopic dermatitis and, if necessary, go through the questionnaires on severity of atopic dermatitis. Further telephone calls are planned at the age of 18, 36 and 48 months.

You can find further information about the study on our homepage at "www.giraffe-study.com". A description of the study is also available under "www.clinicaltrials.gov/ct2/show/NCT04599946".

The study formulas are manufactured in New Zealand by Dairy Goat Cooperative, which has been producing infant formula for Europe and other parts of the world for more than 30 years. The formulas comply with European directives. Both formulas have the same nutritional composition in terms of total contents of energy, protein, carbohydrates and fat. Both formulas are available as infant milk and follow-on milk. Follow-on formula may be used from the age of 6 months onwards or after the start of complementary feeding (feeding of solids). The follow-on milk has the same energy and macro-nutrient content as infant milk, but vitamins and trace elements are adapted to the advanced age and the concurrent intake of complementary foods.

Child's food and drinks

There are no restrictions on food choices for your child. Just follow the advice of your family doctor and national nutritional recommendations. In general, you should start complementary feeding not before the age of 4 months (17 weeks) and not later than at the age of 6 months. When to start complementary feeding depends on your child's development and differs from child to child. As a guideline you can try to start if your

child can sit upright and hold his head up straight, has the oral motor skills to handle solid foods (no direct pushing out of food with tongue), and is interested in beginning and continuing to eat solids.

If possible do not feed other formula or milk than the provided study formula in the first year of life. Please use the study formula also for other foods usually prepared with milk. This will help to guarantee the success of the study.

Benefits and risks when participating in the study

By participating in this study, your child will have the opportunity to consume high-quality formula milk which has been shown to be safe and well tolerated. In addition, we will provide detailed surveillance of your child's growth, development and health and offer additional advice to you on child care and nutrition. With all infant formulas, a few infants develop intolerances. If in doubt, you can ask study team or your paediatrician or family doctor for advice. Besides the free formula, the provision of the blood count, and small gifts for your child when participating in the study visits, there are no other direct benefits by participating in the study. We will reimburse your travel costs for participating in study visits. Your participation will help to improve infant nutrition for future generations.

The risk of blood collection is negligible. It is possible that a local bruise may form and, in very rare cases, infection and inflammation at the puncture site is possible. For the stool samples, there is a minimal risk of contamination when not appropriately using tubes and storage packs.

If important new findings become known during the course of the study that could affect your decision to continue participating in this study, you will be informed immediately. You may then receive a new parental information and consent to sign if you wish to continue participating in the study. In rare cases it may be required to exclude your child from participation in the study for medical or organizational reasons. In this case, we will inform you, delete all personal contact data and use the study health data collected so far without your personal details (see also below).

Laboratory tests

Blood values provide important information to assess the effects of diet on the body. Laboratory analyses include the full blood count, however most blood results are not intended for the individual use as done in the case of illnesses by your paediatrician. The other blood analyses in the study are performed for scientific evaluations only,

and most are determined in a central laboratory with a longer time interval after blood collection. As many children as possible should participate in the blood collection, so that a sufficient number of samples can be obtained to gain meaningful insights, e.g. in relation to the development of allergies! Therefore, we very much hope that you will agree to a blood sample to be taken from your child. The blood samples are used, in addition to health tests (blood count), to measure substances related to allergies (e.g. immunoglobulins, inflammation) or different nutritional and metabolic effects of the formulas (e.g. lipidome, metabolome) that might be related to health, and genetic markers that influence the development of eczema and allergies. We will inform you about the blood count.

The stool samples are used to assess the development of healthy gut bacteria.

All samples are given a code instead of your child's name. This code is a combination of letters and numbers. The code can be related to all other study health data of your child to facilitate the scientific analysis. The code cannot be directly related to your child and ensures personal contact data protection (see below).

Genetic studies

The causes of atopic dermatitis are manifold. Genetics (inheritance) also plays a major role. Studies have shown that the skin protein Filaggrin plays an important role in the barrier function of the skin. Several changes (loss-of-function mutations) in the Filaggrin gene have been identified in patients with atopic dermatitis and are risk factors for atopic dermatitis.

If you agree to the test of the Filaggrin gene, no additional blood sample needs to be taken. The genetic material (DNA) will be extracted from the blood cells, which are left over from the blood sample taken for the other laboratory tests.

However, the Filaggrin gene is not the only risk factor for a child to develop atopic dermatitis or other allergies. Many other genetic and epigenetic factors are involved. The knowledge is constantly increasing. Until recently it was believed that genetic factors, i.e. genes, were simply present or not present, today we know much more about how genes can be "switched on and off". By examining the whole genetic material in the blood (genome-wide genotyping), we can determine which genetic variants may be relevant for the development of atopic dermatitis, related diseases and the metabolism. Furthermore, switching on or off of specific genes is of relevance can be studied (epigenetic investigation). As we are recruiting a very large number of infants in this study, which is a unique and rare opportunity for scientific

advancement, we also would like to take the opportunity to collect material for these analyses.

If you agree to the examination of the whole genetic material, the genetic material (DNA) is obtained from the blood samples of your child and examined. As for the Filaggrin gene test, no additional blood sample needs to be taken, but DNA would be collected at the ages of 4, 12 and 60 months to detect changes in gene expression.

These genetic tests will only be carried out at a later date when samples are available from as many study participants as possible. The examinations of the hereditary factors are carried out at an external institute under the auspices of the key principal investigator (Prof. B. Koletzko, LMU). Double coding (a continuous laboratory number is assigned to the coded samples before processing) prevents the employees of the external institute from drawing conclusions about personal contact data of study participants. This ensures that this particularly sensitive genetic data is additionally protected. Genetic studies are carried out for research purposes only. It is not possible and not intended to communicate results. The statistical analysis of the genetic data is carried out under the responsibility of Prof. B. Koletzko, without reference to the name of your child.

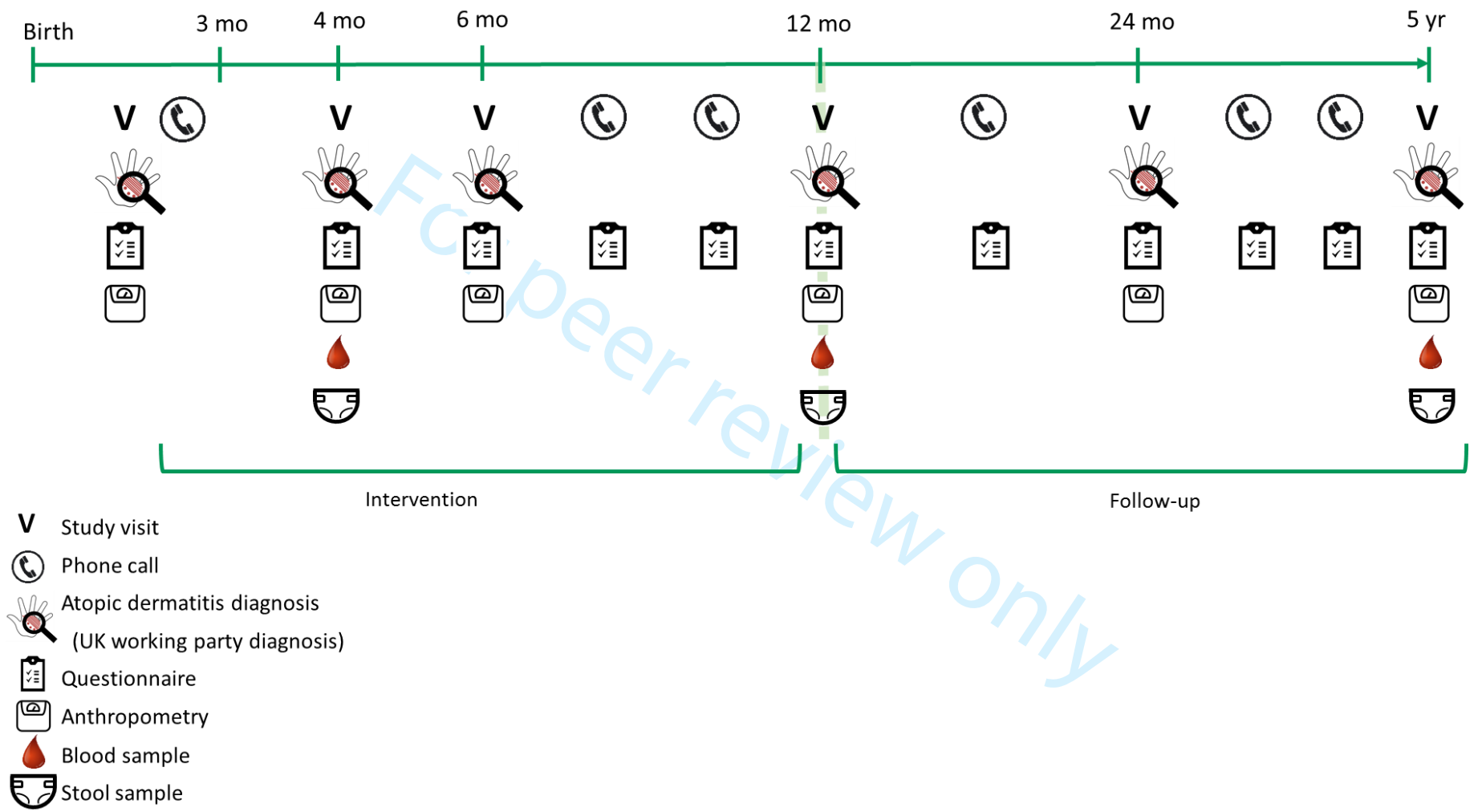
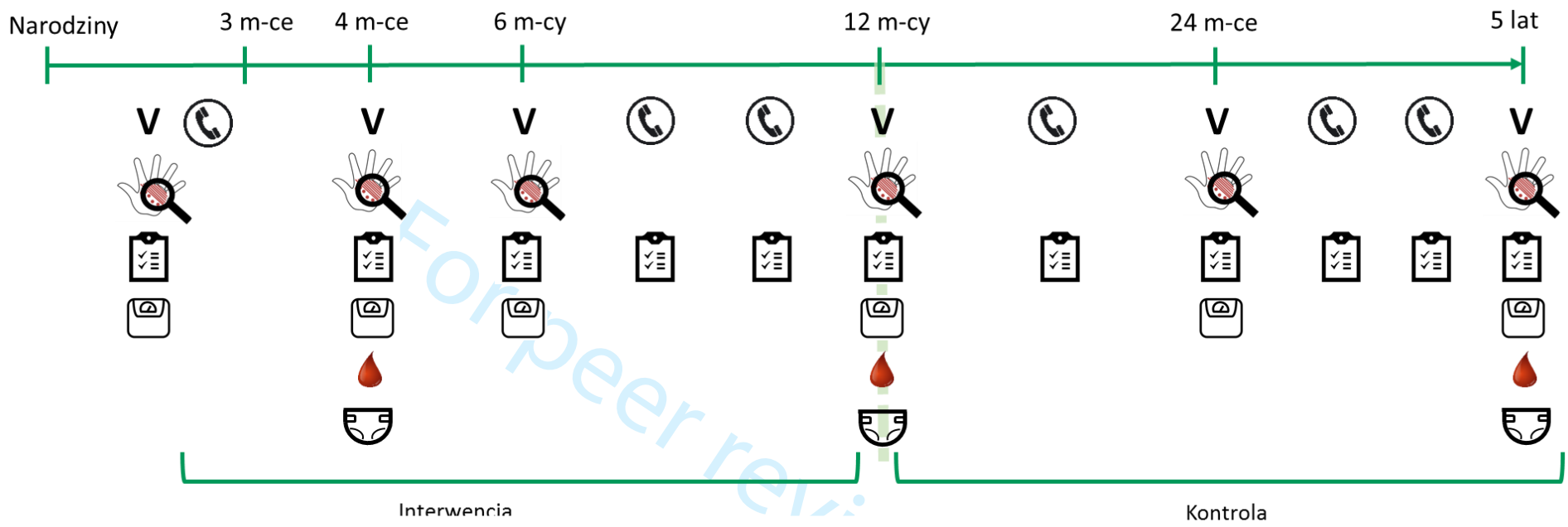


Figure 1. Study plan



V Wizyta w ramach badania

Rozmowa telefoniczna

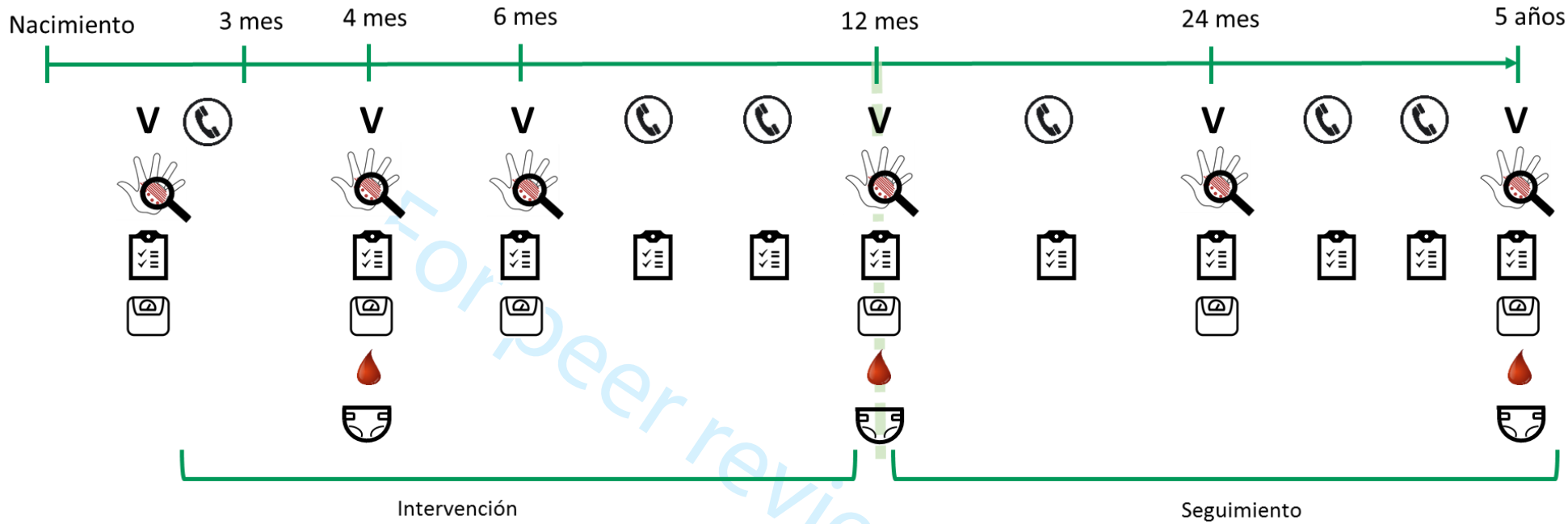
Diagnoza AZS (diagnoza grupy roboczej UK)

Kwestionariusz

Badania antropometryczne

Próbka krwi

Próbka kału



V Visita de estudio

Llamada telefónica

Diagnóstico de dermatitis atópica (DA) (Diagnóstico *UK working party*)

Cuestionario

Antropometría

Muestra de sangre

Muestra de heces

Study evaluation

The data and samples are used exclusively for scientific purposes. The study evaluation is carried out by Prof. Koletzko and his co-workers at the Children's Hospital of the University of Munich, Germany. The data interpretation and publication of study results is carried out by the scientists and medical doctors involved in the study.

Data collected in this study may be used for joint analyses with other studies (meta-analyses), which may include sharing of data with third parties. Double coding of the data as in the genetic analysis described above prevents the employees of the external institute from drawing conclusions about personal contact data of study participants.

After the last follow-up time point of the last subject, we will keep blood samples for up to 5 years to perform all planned analysis. As new insights are constantly obtained in research, we ask you to allow us to keep any excess blood samples during this period, so that blood is not wasted and is still available for possible future, innovative analyses in the context of the study.

In case any excess biomaterials (blood and/or stool) are available after this 5 year period, these will be transferred to a registered biobank (Hauner Biobank, Dr. von Hauner Children's Hospital, LMU, Germany) if you approve. Before transfer to the biobank all data that directly identify you (personal contact data) will be deleted. Your donated biomaterials and the study health data will be made available exclusively for medical research purposes. In order to realize the largest possible public benefit they can be used for a wide range of medical research. The biomaterials and the study health data are intended to be stored and made available for medical research for an undetermined period of time.

All use of data that goes beyond the context of the study will be approved by the ethical committee of the evaluation site.

Study funding

The study is sponsored by Dairy Goat Co-operative (N.Z.) Ltd (18 Gallagher Drive, Hamilton, New Zealand; www.dgc.co.nz; DGC) owned by the farmers who supply goat milk. DGC and the New Zealand Ministry for Primary Industries funded this work as part of the Caprine Innovations NZ (CAPRINZ) Sustainable Food & Fibre Futures Partnership programme. Funding covers the necessary study staff and equipment, all planned aspects of the study, laboratory tests and provision of study formula. Any future

scientific investigations will be carried out with further industrial funding or state support.

Insurance

Although no complications are expected, all study participants are covered by a study insurance. The insurance covers all damage to health that occurs as a result of the measures applied in connection with the study up to a maximum amount of € XXXX.

In case of damage, you can contact the insurer directly (xxx, tel.: xxx; policy number: xxx) and assert your claims. To ensure the insurance cover is not jeopardized, you must tell us all medical treatments that your child undergoes during the study phase (exceptions are preventive examinations and vaccinations). This also applies to the use of new medications. If you or your child have any damage to your health that may have occurred as a result of participating in the study, please inform the relevant study staff and the above-mentioned insurance company.

Voluntariness / Withdrawal Clause

Participation in the study is voluntary. With your signature on the "Consent Declaration" you give your consent to your child's participation in this study. You have the right to stop participating in the study at any time without giving reasons and without disadvantages.

Compensation

For participation in the study you will be compensated for expenses.

If you have further questions about this study or if you think you or your child have suffered a study-related health impairment, we are at your disposal; Tel.: xxx E-mail: GIraFFE.Studie@xxx.xx

Data protection:

The following data protection rules apply as part of the study.

Data protection: This study complies with the rules on medical confidentiality and data protection in accordance with the European and [your countries] directives and the Helsinki Declaration. Your contact details will be stored in a database (MedSciNet, Stockholm, Sweden, http://medscinet.com/). This database only stores personal contact data, but no medical data. In order to deliver the study formula, your contact details are passed on to an external logistics company (xxx). The company is prohibited from using this data for purposes other than the delivery of the study formula. The company is subject to [XXX] statutory data protection regulations.

All other data - i.e. "study health data" - which are not used for contact organisation are stored in separate database (MedSciNet, Stockholm, Sweden, http://medscinet.com/ as well as in the hospital of the University of Munich). Personal contact data such as name or address is not collected in this database. The assignment to your child's name can only be done using a code, which can only be assigned to a name with the active help of the staff at the study center. Thus, all collected data and findings of your child are pseudonymized.

You have the right to receive information about your stored personal contact data at any time, to correct it or, if necessary, to have it deleted.

Responsible for data processing is Prof. [local PI].

Contact details of the data protection officers:

In the event of a complaint, you have the right to contact the respective data protection supervisory authority. For [your institution] this is:

Data Protection Officer

[XXX contact information of local data protection officer]

The higher authority for [your institution] is:

[contact information of a federal or similar higher level data protection officer]

Data access:

Access to personal database with name, contact details, contact information, and ID codes (connecting study health data to personal contact data) is limited to persons involved in the study under supervision of Prof. [local PI]. For organizational reasons and to monitor the study, also personal under the supervision of Prof. B. Koletzko (LMU) will have access to the personal database. Dairy Goat Co-operative Ltd. can commission monitoring of the quality of the study. The monitor (currently Uta Clausen) is committed to data protection and has access to personal and study health data on site. Decoding of individual study participants is only carried out for safety reasons ("medical reasons"). The monitoring company is subject to the local, statutory data protection regulations. All persons with access to the data are listed in a log file and have a personal, traceable login.

Dairy Goat Co-operative Ltd, LMU and the study centres/sites have access to study health data. Dairy Goat Co-operative Ltd. has never access to personal contact data. Study centers only have access to the personal contact data of participants at their site(s). Use of a code will protect your identity and ensure the confidentiality of your data. As data controller, LMU will apply contractual, organizational and security measures ensuring the maintenance of an adequate protection level required by the European and [study site's country] statutory data protection regulations. During those procedures, you and your child identity will not be disclosed.

For the laboratory analyses, the blood samples are only passed on with a code and do not allow any conclusions to be drawn about an individual study participant. The storage of the samples and some laboratory analyses are carried out in laboratories of the hospital of the University of Munich. Genetic analyses and some further examinations are carried out at external institutes. For the genetic analyses, a 2nd encryption by the employees of the external institute is carried out. This double coding ensures that the genetic data is additionally protected. Unblinding is only possible through the study center, but not through the external institute.

In case of withdrawal of consent, the name and your personal contact details will be deleted from our database. Your child's data stored until then will now be used anonymously. In addition, the name and personal contact details of all study participants will be deleted within one month of completion of the study (including analysis of bio-samples). The written documents, including this declaration of consent, will be kept in the study center until the end of the study and in a suitable warehouse until the end of the statutory retention period (12 years after the end of the study).

In the case of publication of the study results, the confidentiality of your child's personal contact data is also guaranteed, as the data is reproduced, if at all, in an anonymized form. On request, we will inform you about general study results. In the event of additional investigations or data collection that go beyond the above-mentioned course of study, we will

Before you enter the study, you have the opportunity to write down specific questions, which should be discussed in more detail with you.

Consent & Privacy Policy for the participation of my/our child in the GIraFFE study

Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis

(GIraFFE study)

Surname, first name of the child	Birth date

The study conditions have been fully explained to me and all questions have been clarified to my satisfaction. I have received the form with the study information. I have had plenty of time to read this form and ask questions. Possible risks and disadvantages for my child were explained to me. I know that I can ask any question about this study and the investigations now and in the future. I know that I/my child can withdraw from the study at any time without having to give reasons or that I or my child would suffer any disadvantages. I hereby consent to my child's participation in the study:

Date	Surname, First name 1. parent or legal guardian	Signature 1. parent or legal guardian

I have sole custody: ☐ Yes ☐ No

Date	Name, Forename 2. parent or legal guardian	Signature 2. parent or legal guardian

Date	Name, Forename Study personnel	Signature

I have taken note of the data protection information within the scope of the participant information. I hereby consent to the collection and use of my child's personal contact data in accordance with these conditions.

Date	Surname, First name 1. parent or legal guardian	Signature 1. parent or legal guardian

Date	Name, Forename 2. parent or legal guardian	Signature 2. parent or legal guardian

Date	Name, Forename Study personnel	Signature Study personnel

Consent & Privacy Policy for the genetic examination of my/our child in the GIraFFE study and for biobanking

Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis

(GIraFFE study)

Surname, First name of the child	Birth date

☐ I hereby agree that genetic material may be extracted, stored and examined from my child's blood. Genotyping in the GIraFFE study is used to identify a possible genetic cause of a **modified skin protein (filaggrin)** that may be related to the appearance of atopic dermatitis (eczema). Participation in the examination does not involve any further health risks beyond the blood collection.

☐ I hereby agree that genetic material may be extracted, stored and examined from my child's blood. The **genome-wide genotyping and epigenetic investigations** serve to uncover the genetic causes of diseases and causes of allergies and metabolic changes within the GIraFFE study. Participation in the examination does not involve any further health risks beyond the blood collection.

☐ I hereby agree that any excess bio-samples are transferred together with anonymized study health data to a registered biobank as described in the study information.

The data and results will be used exclusively as outlined in the subject information. Only authorized employees of the study can access the encrypted data. Data will not be passed on to unauthorized third parties. The genetic data obtained in the course of this study shall be retained for up to 10 years after completion of the scientific study or until revocation has been made.

I know that I can ask further questions now and in the future about this study. I know that I can withdraw from voluntary participation in the study at any time without having to give reasons. I voluntarily consent to the collection, processing and use of personal contact data in accordance with the information sheet of the study.

Date	Surname, First name 1. parent or legal guardian	Signature 1. parent or legal guardian

Date	Surname, First name 2. parent or legal guardian	Signature 2. parent or legal guardian

Date	Surname, First name Study personnel	Signature Study personnel

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	✓ various pages
Protocol version	#3	Date and version identifier	13
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 2, 14, 15
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1, 2
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team,	14

		and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3, 4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3,4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data	1, 2, 5

		will be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5, 6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6, 7, suppl. material
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a no
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical	7, 8

		relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9, 10,11
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and	9, 10, 11

		validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7, 9, 10
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a not planned
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	13

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	13
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12

Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a not yet decided
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a not yet planned
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental Material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

BMJ Open

Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis: Protocol of the randomized controlled Goat Infant Formula Feeding and Eczema (GIraFFE) trial

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Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis: Protocol of the randomized controlled Goat Infant Formula Feeding and Eczema (GIraFFE) trial

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Abstract

Introduction Atopic dermatitis (AD) is a chronic, inflammatory skin condition significantly affecting quality of life. A small randomized trial showed an approximately 1/3 lower incidence of AD in goat milk formula-fed compared to cow milk formula-fed infants. However, due to limited statistical power AD incidence difference was not found to be significant. This study aims to explore a potential risk reduction of AD by feeding a formula based on whole goat milk (as a source of protein and fat) compared to a formula based on cow milk proteins and vegetable oils.

Methods and analysis This two-arm (1:1 allocation), parallel, randomized, double-blind, controlled nutritional trial shall enrol up to 2296 healthy term born infants until 3 months of age if parents choose to start formula feeding. Ten study centres in Spain and Poland are participating. Randomized infants receive investigational infant and follow-on formulas either based on whole goat milk or on cow milk until the age of 12 months. The goat milk formula has a whey:casein ratio of 20:80 and about 50% of the lipids are milk fat from whole goat milk, whereas the cow milk formula, used as control, has a whey:casein ratio of 60:40 and 100% of the lipids are from vegetable oils. The energy and nutrient levels in both goat and cow milk formulas are the same. The primary endpoint is the cumulative incidence of AD until the age of 12 months diagnosed by study personnel based on the UK Working Party Diagnostic Criteria. The secondary endpoints include reported AD diagnosis, measures of AD, blood and stool markers, child growth, sleep, nutrition and quality of life. Participating children are followed until the age of 5 years.

Ethics and dissemination Ethical approval was obtained from the ethical committees of all participating institutions.

Trial registration number NCT04599946 (registered on 23.10.2020)

Strengths and limitations of this study

- Potential confounding is minimized due to the randomized study design.

- A multicentre study design with sites in different countries increases external validity of study results.
- The follow-up until five years of age allows to examine long-term effects of infant feeding.
- Effect sizes may be limited due to the short-time period of consuming study formula as the only food.

Keywords

Goat milk; infant formula; clinical trial; atopic dermatitis; eczema; infant nutrition; food allergies; cow milk.

Word Count: 4123

INTRODUCTION

Atopic dermatitis (AD), also known as eczema or atopic eczema, is a chronic, inflammatory, pruritic skin condition that frequently occurs in children [1] and adults. It is characterized by intense itch, recurrent eczematous lesions, and a fluctuating course. AD affects 15 to 30% of children in industrialized countries [2]. The highest frequency of AD onset is reported for the first year of life, but it can start in later phases of childhood and even in adult age [3 4]. It is reported to often be the prelude to an atopic march including food allergies, asthma, and allergic rhino-conjunctivitis [5].

The strongest risk factor for AD is a positive family history of AD and atopic diseases in general, with a 4.7-fold risk increase if both father and mother were affected by AD [6]. This is in agreement with the identification of 34 specific genomic regions that seem associated with AD susceptibility, including the strongest genetic risk factor for AD, the semi-dominant null mutations in the Filaggrin gene [7]. This gene encodes the epidermal protein Filaggrin and the mutation causes a reduction in Filaggrin expression [7]. Further factors influencing AD risk include climate, place of residence, household pets, diet, prolonged breastfeeding, obesity, physical activity, pollution, day care attendance, basic hygiene, family size, infections in childhood, applications of antibiotics and use of emollients [8].

The clinical phenotype observed in individuals with AD is variable. To support diagnosis, several sets of criteria considering the intermittent nature of AD and possible fluctuations in AD activity, have been developed including the UK Working Party criteria [9-11]. Validated scoring systems such as the Scoring Atopic Dermatitis (SCORAD) or the Patient-Oriented Eczema Measure (POEM) have been introduced [12-15].

Taking the considerable loss of quality of life[16] and associated disease risks in children affected by AD into account, infant feeding schemes for the general population associated with a decreased risk of AD manifestation would be highly desirable. So far, no generally accepted strategies for primary

prevention of AD are available. For infants at high risk of developing AD, a 4-month period of breastfeeding might be advisable, but results are controversial [17]. Formulas based on hydrolyzed proteins, as well as prebiotics and probiotics, were reported to provide protective effects but results are inconsistent [18-20].

Because of cross-reactivity, goat milk proteins can induce reactions in infants allergic to cow milk proteins, which precludes the recommendation of goat milk protein-based formulas for infants allergic to cow milk protein [21]. Nevertheless, there are indications from animal studies that goat milk is less allergenic than cow milk [22 23] although such differences are not confirmed in all studies [24]. The allergenic protein αs1-casein is the dominant casein in cow milk, with 12–15 g/L. In contrast, goat milk has variable levels of this protein dependent on the genotype of the goats, ranging from 0.9 to 7 g/L. In addition, caseins from goat milk are broken down to a greater extent than those from cow milk during digestion, corresponding to a potentially lower allergenic burden from goat milk [25]. Although there is a 88% sequence homology between cow and goat αs1-casein, a recent study in mice found the goat milk protein less sensitizing than the cow milk protein [26].

A multicenter, double-blind, controlled feeding trial in Australia, found that an infant formula based on cow milk proteins (n=101) and vegetable oils and a formula based on whole goat milk (n=99) were both well tolerated and supported physiological growth comparable to breast fed infants (n=101) [27]. This is in agreement with two other studies performed in New Zealand [28] and China [29], which tested formulas based on whole goat milk and goat milk protein, respectively. The Australian study included assessment of dermatitis using SCORAD and found an incidence of 23% in the cow milk formula group compared to only 14% in the goat milk formula group [27]. Although this corresponds to an approximately 1/3 lower incidence of AD, the difference was not statistically significant (Fisher´s Exact test) given that the study was powered to evaluate potential growth differences, but not differences in AD incidence between groups [27]. The addition of cow milk fat globule membranes to infant formula had shown positive effects on the neurological development of the infants and a decreased use of antipyretics, which could indicate less inflammation [30 31]. In a murine model of AD inclusion of goat milk lipids into the diet had reduced inflammation [32]. The complexity of goat milk lipids, including sterols, sphingolipids, and glycerophospholipids, seems similar to cow milk lipids [33 34]. The different proteins and polar lipids in the formula let us expect effects on the plasma metabolome and the gut microbiome as suggested by previous human and animal studies, respectively [35-37]. These biomarkers might enable mechanistic insights into associations between infant diet and the risk of AD development.

Therefore, the Goat Infant Formula Feeding and Eczema (GIraFFE) study tests whether infant feeding with a formula based on whole goat milk (protein and fat) reduces the risk of developing AD when

compared to a formula based on cow milk proteins and vegetable oils. Secondarily, the study aims to contribute to the identification of risk factors for AD, elucidation of the mechanistic understanding of the immune system development and to provide a resource for studying other questions related to infant nutrition and development.

Primary Objective

The primary objective of this trial is to determine the relative risk of developing AD in the first 12 months in infants fed a formula based on whole goat milk compared to infants fed a formula based on cow milk.

Secondary Objectives

The secondary objectives are related to AD and other atopic diseases but also to the child´s growth and wellbeing, including infant metabolism and gut health, in the first 5 years of life. All outcomes will be compared for an effect of the study formula treatment (goat formula vs cow formula). The study will also explore associations of AD and other atopic diseases and overall development, and aims to identify risk indicators.

METHODS AND ANALYSIS

Study design and population

The GIraFFE study is a randomized, double-blind, parallel-group, superiority clinical trial to study the effect of feeding infants a whole goat milk or a whey-adjusted cow milk formula during the first year of life on the risk of allergy and other health outcomes, including growth and quality of life, in the first 5 years of life. The study is led by the key principal investigator Professor Dr. Berthold Koletzko and conducted as a multicentre trial in currently 4 study centres in Poland and 6 study centres in Spain, which all have local principal investigators.

The study population consists of healthy term infants of parents who decided to start formula feeding, without a preselection for children with an increased risk for AD. The study teams proactively promote, support and protect breastfeeding. Only infants of parents who decided to start formula feeding are enrolled into the study but are encouraged to continue partial breastfeeding after enrolment. The infants participating need to fulfil the criteria depicted in Table 1.

Table 1: Inclusion and Exclusion criteria of the GIraFFE study

Inclusion criteria	Exclusion criteria

• Written informed consent (signed and dated) of the child's parent(s)/caregiver(s), indicating that the child's parent(s)/caregiver(s) has/have been informed of all pertinent aspects of the study • Infant was born full term (≥37 weeks +0 days and ≤ 41 weeks +6 days of gestation) • Age at enrolment < 90 days • Infant birth weight ≥2.5 kg and ≤4.5 kg • Infant is born from a singleton pregnancy • Child's parent(s)/caregiver(s) is/are of legal age of consent • The child's parent(s)/caregiver(s) have sufficient local language skills to understand the study information, the informed consent, and to comply with the study procedure • The child's parent(s)/caregiver(s) is/are willing and deemed able to fulfil the requirements of the study protocol and procedures • Mother has expressed the intention to partially (in combination with breastfeeding) or fully formula-feed	• Diagnosed disorder considered to interfere with nutrition, growth, or development of the immune system • Participation of the child in any other interventional trial or participation of the mother in any intervention trial with child follow-up • Infant has a doctor's diagnosis of AD or a severe widespread skin condition prior to randomization that makes the detection or assessment of AD difficult • Infant has regularly (on average at least 3-4 days a week, at least one bottle per day) consumed an infant formula other than study formula for more than 4 weeks prior to enrolment • Cow's milk allergy or intolerance of the child • Institutionalized infant

Study formulas

Participants are randomly assigned to receive one of the two formulas manufactured by Dairy Goat Co-operative (N.Z.) Ltd (Hamilton, New Zealand). The goat milk formula is already marketed as Capricare; it is based on whole goat milk as a source of protein (20:80 whey:casein ratio) and goat milk fat contributes 50% of total fat. The control formula is based on cow skim milk and whey protein powders (60:40 whey:casein ratio) and vegetable oils as the almost only source of fat. The study

formulas are isocaloric, have the same macronutrient composition and are provided as infant and follow-on formulas (Supplementary Table 1). The composition of all formulas complies with European Commission Delegated Regulation (EU) 2016/127.

The key differences are 1) the source of milk from cows or goats, 2) the whey:casein ratio, and 3) the fat source.

Study product intake and compliance

Feeding of study formulas can begin immediately after enrolment, but must start no later than the age of 4 months and continues until the age of 12 months. The study formula is fed ad libitum and shall be the only formula given to the participating infant. If infants do not consume at least some study formula before the infant is 4 months old, the infants are excluded from the study.

Preparation and feeding guidelines are identical for both study formulas and are in agreement with common practice. The study teams advise not to use follow-on formula prior to the infant age of 6 months, but it is the parent's decision whether and when to introduce follow-on formula. Compliance is defined as a continuous study formula consumption over the whole intervention period without any breaks longer than 3 consecutive days and no introduction of solid foods before the age of 4 months. Compliance will be checked at all scheduled study contacts and plausibility of continuous consumption will be checked by the number of consumed cans.

Outcome measurements

The primary endpoint of the GIraFFE study is the cumulative incidence of AD up to the age of 12 months diagnosed by study personnel, defined as meeting the UK Working Party Diagnostic Criteria for AD. The secondary endpoints are listed in Table 2.

Table 2: Secondary endpoints of the GIraFFE study

Secondary endpoints	**Time frame (age)**
Cumulative incidence of study personnel-diagnosed AD, defined as meeting the UK Working Party Diagnostic Criteria for AD	up to 24 and 60 months
Cumulative incidence of parental reported diagnosis of AD defined as meeting the UK Working Party Diagnostic Criteria for AD, in a telephone interview or parental report of a	up to 12, 24 and 60 months

non-study doctor diagnosis in addition to the study diagnosis of AD	
Point incidence of study diagnosed and parental reported AD, defined as meeting the UK Working Party Diagnostic Criteria for AD	at 4, 6, 12, 24 and 60 months
Age at first study diagnosis, parental report-based study diagnosis or parental report of a diagnosis of AD by a non-study doctor	up to 12, 24 and 60 months
AD severity in children with diagnosed (study diagnosis or reported diagnosis) AD, using SCORAD questionnaire completed by study personnel at all face-to-face visits	4, 6, 12, 24 and 60 months
AD severity in children with diagnosed (study diagnosis or reported diagnosis) AD, using POEM questionnaire completed by parents at all scheduled contacts	4, 6, 8, 10, 12, 18, 24, 36, 48 and 60 months
Cumulative use of eczema-related medication or skin care	up to 12, 24 and 60 months
Parental report of a clinical diagnosis of food allergy	12, 24 and 60 months
Parental reported hay fever and asthma-related diseases	up to 12, 24 and 60 months
Anthropometric measures (weight-for-age, length-for-age and BMI-for-age z-scores) at baseline	at 4, 6, 12, 24 and 60 months
Parental report of gastrointestinal symptoms (Infant Gastrointestinal Symptom Questionnaire, IGSQ) and sleep (Brief Infant Sleep Questionnaire, BISQ	at 4, 6 and 12 months
Quality of life in children using the Infant Toddler Quality Of Life questionnaire (ITQOL) filled by parents	at 4, 12, 24 and 60 months
Nutrition questionnaire	at 4, 6, 8, 10, 12 and 60 months
Allergic sensitization (total and specific IgEs including cow milk protein and goat milk)	at 12 and 60 months

Blood lipids, metabolome, lipidome and further exploratory markers	at 4, 12 and 60 months
Gut microbiome	at 4, 12 and 60 months

Sample size

The number of subjects to be studied was based on the incidence of AD in the population and the effect size to be detected. Reported AD incidence estimates for young children in Spain and Poland are 13% and 17%, respectively [38 39]. The previous study comparing goat and cow milk formulas had indicated a risk reduction for AD incidence of 30% [27]. Thus; we assume a cumulative incidence of AD at 15% in the first 12 months of life, based on the cited data, and a 30% clinically relevant risk reduction by whole goat milk formula. A sample size of 861 infants per group is required to set the significance level to 0.05 and statistical power to 80%. We estimate the dropout rate until the age of 12 months to be 25%. Thus, 1148 infants per group (in total 2296) need to be studied. If the dropout rate turns out to differ markedly from the assumption, the number of infants to be recruited may be adjusted during the study.

Recruitment

Precautions are taken to ensure that recruitment does not undermine breastfeeding intentions and practice. Due to differences in health care systems and local infrastructure the way to approach and recruit subjects is different for each study centre. In most cases those families who expressed their decision to partially or fully formula-feed are made aware of the study in paediatric practices or primary health care centres. In any case, parents are not informed about the study until they had decided to feed the baby with formula or both formula and breastmilk, in order not to interfere with breastfeeding. The recruitment of study participants has started in January 2021 and is currently ongoing in all ten study centres.

Blinding and randomized allocation of study formulas

The study is double blinded using four different 3-character codes, two for each study product. Study personnel, biostatistician, data manager, trial monitor, laboratory analysts, and all persons involved in the organization and conduct of the study and study participants are blinded. Study products are shipped to the participating families and the sites by logistic partners.

For the allocation of the subjects to the four study codes minimisation randomization (1:1:1:1 ratio) is applied with centres as the only strata [40 41]. The dynamic randomization method minimizes imbalances

in age at randomization and sex. A random element makes assignment unpredictable with a maximal group difference of +/- 4 children allowed. The randomized allocation sequence is provided as part of the study management tool by CSAM MedSciNet U.K. Ltd. (Reading, UK) based on a published procedure [42].

DATA COLLECTION, MANAGEMENT AND ANALYSIS

Data collection and management

Figure 1: Schematic representation of the study design

During the intervention, study centre visits are planned at enrolment (= baseline and randomization), at 4, 6 and 12 months of age, and during the follow-up visits at 24 and 60 months (Figure 1). Telephone contacts after enrolment and at 2 and 3 months of age are done, depending on the age at enrolment. After the face-to-face visits at age 4 and 6 months, which aim to collect data during the phase of dominating formula feeding (4 months) and the age of high incidence of AD in the Australian study at age 6 months [27], phone calls are scheduled at age 8 and 10 months for further data collection and to support protocol compliance and study logistics during the intervention period. During the follow-up, telephone calls are performed at 18, 36, 48 months of age to collect data and enhance contacts with the participating families.

An initial screening for eligibility is performed at the first contact with potentially participating families, and at the enrolment visit prior to randomization the subject´s suitability according to inclusion and exclusion criteria is confirmed. Families willing to participate sign the informed consent form. A template informed consent form is enclosed in the supplemental material. At the baseline visit information about atopic diseases of parents and siblings, pregnancy information, birth data, socio-economic background, the home environment, the child´s medical history and details of feeding practices since birth are collected. At all study visits anthropometric measurements are performed.

The UK Working Party Diagnostic Criteria are used for AD diagnosis at enrolment and at all subsequent visits and telephone calls until the age of 60 months. Criteria are adapted for children under the age of 12 months in respect to time frame and body areas considered and at the telephone calls, when no direct visual inspection is possible and parental report at the visit day are documented.

Two questionnaires are used for the assessment of the severity of diagnosed AD (study diagnosis or reported diagnosis): 1) SCORAD questionnaire at all face-to-face visits 2) POEM questionnaire at all examination time points up to 60 months of age. For all children who were ever study-diagnosed with AD, this reflects the objective view of trained medical personnel (SCORAD) [13 14] and the more subjective view of the parents (POEM) [12 15].

The introduction of complementary feeding, use of cow milk and cow milk products, allergenic foods, use of beverages and food preferences is assessed with questionnaires at 4, 6, 8, 10 and 12 months. At the 12-month and 60-month visits, Food Frequency Questionnaires (FFQ) are used for a more detailed assessment of dietary habits. The FFQ was modified according to the age of children, based on an FFQ applied in the Identification and prevention of dietary- and lifestyle-induced health effects in children and infants project [43].

During all telephone calls and visits in the intervention period, intake and acceptance of the formula is assessed as compliance indicator. For adverse event recording, participating families are asked in all scheduled visits and telephone calls for hospitalizations, illness and any medication of the child.

Parents are explicitly asked for a doctor's diagnosis of food allergies at 12, 24 and 60 months with a specific focus on cow milk, egg, peanuts, soy, and fish. Furthermore, asthma, bronchitis/bronchiolitis, wheezing and allergic rhinitis at the 60-month visit with distinction between self-observation and doctor-diagnosis are assessed.

During the intervention period, questionnaires about sleep (BISQ) [44] and gastrointestinal problems (IGSQ) [45] are applied at the face-to-face visits. Furthermore, the Infant Toddler Quality Of Life questionnaire (ITQOL) [46] is completed at 4, 12, 24 and 60 months by parents. Parents are asked at enrolment, and at all contacts from 4 months on about general skin care and if there has been a

prescription of topical treatment like corticosteroid or other immunosuppressive therapies by a physician since last visit.

Data are collected primarily with a web-based online database developed by CSAM MedSciNet U.K. Ltd (Reading, UK) with direct data entry by study personnel and participating families as default option. The use of paper forms is limited to situations where the direct input into the database is technically not possible or not wished by parents. Furthermore, copies of signed consent forms are stored electronically. All procedures are checked for general data protection regulation conformity by a LMU data protection officer.

Biosamples

Blood collection is planned at the 4, 12 and 60-month visits. Standard operating procedures are in place. Highest priority is given to the analysis of atopy-related parameters such as total IgE, specific IgEs for cow and goat milk protein, as well as further frequent allergens, and inflammation markers. Serum lipids (total cholesterol, HDL-cholesterol, triglycerides) and lipidomic and metabolomic analyses aim at describing the metabolism of the infants in respect to formula consumed and for the identification of biochemical risk markers or eventual metabolic consequences of AD. As a safety indicator, full blood count is taken from all blood samples. If corresponding consent has been obtained, Filaggrin genotype will be determined and further genetic analysis performed if additional funding is granted.

For microbiome analyses, stool samples are collected at 4, 12, and 60 months in a subgroup of 600 infants. At enrolment, interested families receive the stool collection material as well as written instructions. A questionnaire is used to record the classification of the stool sample on the Brussels Infant and Toddler Stool Scale and the administration of probiotics. According to the standardized procedures, samples should be frozen at -20°C within less than 15 min after collection; samples have to be transferred to a -80°C freezer within a week. The details of microbiome analyses have not been fully defined yet, but will apply established DNA extraction and amplification methods and corresponding bioinformatics tools.

Adverse Events

Adverse events (AEs) are recorded according to a standardised protocol including an opinion on the assumed relation to the intervention and a categorization of the AEs. During the intervention and until 30 days after study product intake all safety events fulfilling the following criteria are reported as AEs.

- Child was treated with:
    - Medication > 14 days

- Oral antibiotics
- Inhalation therapy
- Steroids, salbutamol, antihistamines, montelukast

- Child was hospitalized
- Child was treated with a special diet > 7days
- Child interrupted the intake of the study product > 1 day or completely discontinued consumption

From 31 days after the last product intake only safety events, fulfilling the applicable criteria, and considered as potentially related to the intervention or that may influence study outcomes are reported as AE.

Any AE that results in death, is life-threatening, requires hospitalization or results in persistent or significant disabilities is classified as serious adverse event (SAE). The Principal Investigators of the individual study centres review all SAEs at their centre and provide an opinion, including a comment on the relation to the intervention.

A clinical trial insurance has been set up.

Monitoring

An external monitor performed the study monitoring during the first ten months of the study recruitment. After this period monitoring activities were taken over by LMU researchers. Monitoring should improve the quality of the collected data but mainly focuses on the compliance of all local study procedures with the protocol, established SOPs and good clinical practice. Besides on-site monitoring, additional remote monitoring is also performed.

A Data and Safety Monitoring Board (DSMB) has been established, with the primary responsibility of reviewing and evaluating data for participant safety and study progress including a critical review of the findings after the first 128 participants have completed the intervention period. The DSMB review focuses on interim/cumulative data of study-related adverse events, individual center performance, protocol deviations and external factors such as scientific or therapeutic developments that may have an impact on participant safety or raise ethical concerns. Based on the accumulated study data, the board makes recommendations concerning continuation, modification, or eventual termination of the GIraFFE study.

The DSMB consists of three members who have no direct involvement in the conduct of the study, financial, professional, or other interests that may affect independent decision-making.

If the recruitment rate is less than 50% of the expected rate after 12 months, or if the primary objective yields no effect of the intervention, the study may be terminated prematurely. After consulting with the trial steering committee, the sponsor and key PI will decide about the premature study termination.

Statistical analysis

Statistical analyses are scheduled when all recruited infants have passed V12, V24 and V60, respectively. All primary and secondary analyses including methods to deal with missing data and sub-group analyses are to be specified in a Statistical Analysis Plan, which is finalized prior to database lock and unblinding.

As primary statistical analysis, a comparison of the cumulative incidence of children with AD until 12 months of age between the goat milk formula group and cow milk formula group is planned. For this analysis, a Generalized Estimating Equation Poisson model with a log link and robust standard errors by sandwich estimators of variance will be used [47].

The findings are compared with further adjusted models that include major influencing factors of AD frequency, including country, sex, Filaggrin genotype, parental atopic diseases, parental AD, antibiotic usage, family size and socio-economic status. Furthermore, interactions of Filaggrin mutations, the number of immediate family members with AD or other atopic disease with AD frequency shall be investigated. If effect modification by one of the mentioned predictive covariates is significant at the 5% level, subgroup analyses for each category will be presented.

Secondary analyses will look at the secondary objectives with similar statistical approaches.

Ethics and Dissemination

Ethical approval was obtained from the ethical committee of the LMU University Hospital Munich, Germany (Nr. 20-188; ethikkommission@med.uni-muenchen.de) and the ethical committees of all ten study centres: Hospital Universitario La Paz, Madrid (Ref. 47/322688.9/20; Ref: 47/748801.9/21); CEIC Aragón (CEICA), Zaragoza (C.P. - C.I. PI20/098); Hospital Clínico Universitario de Valencia Ref. CEIm 2020/219); Institut d'Investigació Sanitària Pere Virgili, Reus/Tarragona (Ref. CEIM: 057/2020); CEIM/CEI Andalucía, Delegación Provincial de Granada (Ref. CEIM/CEI: 1134-M1-20); Hospital Universitario Torrecárdenas, Almeria (Ref. CEIM: 109/2019), Warmińsko – Mazurskiej Izbie Lekarskiej w Olsztynie (Nr 1/2020/VII); Poznań University of Medical Sciences (No 436/20); University of Rzeszów (No. Number 05/07/2020); Instytucie "Pomnik-Centrum Zdrowia Dziecka" (12/KBE/2020).

Currently protocol version 1.1 is valid since 25.06.2020. The ethical committees will approve all protocol amendements prior to implementation.

Patient and Public Involvement

The protocol for the study including all procedures related to subject safety and protection of personal data was predominantly developed at a public hospital, but without specific patient consultations.

Public dissemination and data availability

Researchers and sponsor are committed to publish the study findings in peer-reviewed international scientific journals. Dissemination of study results may also include posting of a synopsis online, abstracts submitted to and presentations at scientific conferences, and other dissemination activities including social media.

After a delay period for full scientific evaluation, the remaining biosamples and associated data of participants, for whom respective consent is available, will be transferred into a registered biobank (Hauner biobank, LMU München). Data and samples will be accessible for other researchers according to the biobank regulations.

Funding, role of the sponsor and investigators

The study products are manufactured and provided to participants by the study sponsor (Dairy Goat Cooperative (N.Z.) Ltd, Hamilton, New Zealand). The sponsor has allocated a fixed budget to each of the institutions hosting the study centre and the key principal investigator with his team to conduct the study.

The sponsor, the site principal investigators and the key principal investigator have agreed and fixed in the study protocol that the final decision making power on the study rests with the trial steering committee, which includes the key principal investigator, all site principal investigators, and the sponsor. The trial steering committee also takes decisions on further grant applications to fund additional analyses of data and biosamples generated in GIraFFE.

Author Contributions Statement

The conception and design of the study was developed by BK, VG, HD, UH and SG. JF produced the first draft of the manuscript and all co-authors RGM, CC, MSP, EJC, JW, BR, JE, MGL, PG, DG, II, VG, HD, UH, SG and BK critically reviewed the manuscript and approved the final version. RGM, CC, MSP, EJC, JW, BR, JE, MGL, PG, DG and II participated in the set-up of the study.

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Conflict of interest

SG is an employee of DGC (Dairy Goat Cooperative (N.Z.) Ltd, Hamilton, New Zealand).

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Colin Prosser (DGC), Philipp Schwarzfischer and Sandra Unterschemmann (LMU) contributed to the development or the implementation of the protocol.

Supplemental Material

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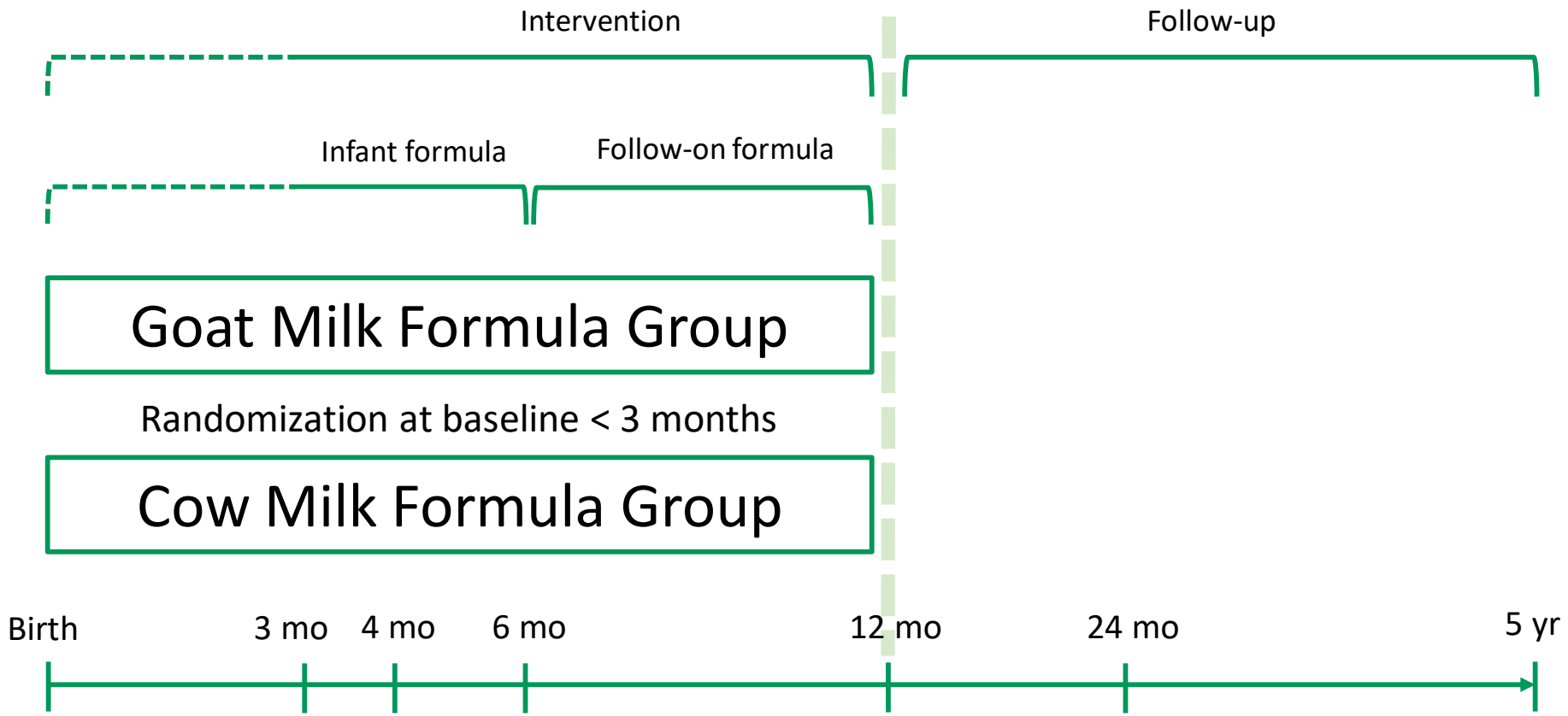

Intervention
Follow-up
Infant formula
Follow-on formula
Goat Milk Formula Group
Randomization at baseline < 3 months
Cow Milk Formula Group
Birth
3 mo
4 mo
6 mo
12 mo
24 mo
5 yr

Supplemental Material

Supplementary Table 1: Composition of the study infant and follow-on formulas:

Nutrients	Unit	**Whole goat infant formula** per 100 mL of prepared feed*	**Cow infant formula** per 100 mL of prepared feed*	**Whole goat follow-on formula** per 100 mL of prepared feed*	**Cow follow-on formula** per 100 mL of prepared feed*
Energy	kj	270	270	270	270
	kcal	65	65	65	65
Fat	g	3.4	3.4	3.4	3.4
Milk fat	% total fat	46	2	46	2
Saturated fatty acids	g	1.3	1.3	1.3	1.3
Docosahexaenoic acid (DHA)	mg	15.5	15.5	15.5	15.5
Arachidonic acid (ARA)	mg	16.1	16.1	16.1	16.1
Carbohydrate	g	7.3	7.3	7.3	7.3
Sugars (lactose)	g	7.1	7.1	7.1	7.1
Protein	g	1.3	1.3	1.3	1.3
Whey	% total protein	20	60	20	60
Casein	% total protein	80	40	80	40

* Prepared as follows: 4.3 g of powder + 30 mL of water (12.9 g of powder per 100 mL of milk).

[1]Retinol equivalents, [2]alpha-tocopherol equivalents, [3]dietary folate equivalents

Parent information and consent

Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis (GIraFFE Study)

Study registration:

NCT04599946 at clinicaltrials.gov

Please read this information carefully. The study staff will answer any questions you may have.

The GIraFFE study was examined by the Ethics Committee and the Data Protection Officer of [your institution's approving committee/person] and obtained a favourable opinion.

You will receive a copy of this letter for your records.

PREAMBLE: *Exclusive breastfeeding is the ideal and healthiest way to feed your infant. This study is only offered to families whose children are completely formula-fed or whose parents have decided to provide mixed feeding (breastfeeding combined with formula). If your infant is receiving mixed feeding, but you would like to achieve exclusive breastfeeding, we can offer you support to achieve this, instead of participating in this study.*

Dear Family,

Thank you for your interest in our study that we are conducting together with partners in Spain, Poland and Germany. We want to find out if nutrition in early life affects the onset of atopic dermatitis (also known as eczema and atopic eczema). The study is called the GIraFFE study (**G**oat **I**nfant **F**ormula **F**eeding and **E**czema).

Why are we performing the study?

The increasing number of children with atopic dermatitis and allergies is a major medical problem. We are interested in understanding why some children develop allergies. Atopic dermatitis affects all age groups, but can be a particular concern for infants and small children. In a small study in Australia, infants were fed either a goat milk-based formula or a cow milk-based formula. A difference in the number of infants developing atopic dermatitis in first year of life was found. While goat milk and cow milk formula are both suitable for infant feeding if breastfeeding is not possible, they slightly differ in their composition, types of fat and proteins. These differences in composition might play a role in the development of atopic dermatitis or allergies. Understanding the role of these factors in the development of atopic dermatitis and allergies will help to choose the most suitable formula, and to improve formula composition and guidelines for infant nutrition.

Purpose of study

The aim of the GIraFFE study is to compare if formulas based on goat milk and cow milk have different effects on the development of atopic dermatitis and other related allergic diseases in a larger number of babies. The study formulas have the same composition of the essential nutritional components to support normal growth and

development of infants. Furthermore, we will assess and analyse stool bacteria and bio samples in the participating child as well as exploring other indicators of general health, development and metabolism.

Course of the study (see also Figure 1)

If you agree to participate, your baby will randomly be assigned to receive the cow or the goat milk formula. This randomization is important to exclude that any other factors related to food choice might cause a difference in eczema occurrence. To prevent any potential influence on the study results, neither you nor the study personnel will know which of the formulas your baby receives during the study. You will receive formula free of charge from enrolment until the study visit scheduled around your child's first birthday (age 12 months). After your child's first birthday, the formula supply will end and you will be free to choose what to feed your child, but the study itself will continue with following your child until the 5th birthday (age 60 months). A total of 2296 infants will participate in the GIraFFE study (distributed over 10 study centres in Poland and Spain). The overall study coordinator is Prof. B. Koletzko at the Dr. von Hauner Children's Hospital at the University of Munich, Germany. The local coordination will be done by XXX.

Participation in the GIraFFE study begins during the first three months of life. After the enrolment examination, further appointments for your child are planned here at [your institution] at the age of 4, 6, 12, 24 and 60 months. Every time you visit us, we will examine your child for signs of atopic dermatitis and measure height and weight. We will ask you questions about your child's health and general behaviour. In order to find out about the environment your child grows up in, we will initially ask you about your origin, education and family structure as well as cases of atopic dermatitis and allergic diseases in the family. In order to understand what your child eats and drinks apart from the study formula, we will ask you at each appointment which complementary foods you have already introduced to your child. At the age of 12 months, we also ask a little more about your child's dietary habits. During the first year of life, we also ask about the sleeping habits (BISQ questionnaire) and gastrointestinal comfort (IGSQ questionnaire) of your child. To assess the quality of life of your child, we will ask you to fill a slightly longer questionnaire (ITQOL questionnaire) at 4, 12, 24 and 60 months of age. Most of the questionnaires will be available online, so will be able to fill them online at home and reduce the time needed for each study visit.

If your child shows signs of atopic dermatitis, we record the severity with a standardized tool called SCORAD, when your child comes in for the next scheduled appointment. In addition, the POEM questionnaire is used to record the influence of atopic dermatitis on your child's quality of life.

In case you agree, we would like to take a small volume of blood (approx. 4-6 ml) from your child by a doctor or trained nurse during visits at 4, 12 and 60 months. We will be happy to share some of the results relevant for the assessment of your child's health such as blood count with you and your paediatrician. For blood sampling we offer applying local anaesthetic cream to the puncture site to avoid inconvenience for your child. At the same time points, we also ask you to collect some stool of your child. A kit for stool collection and instructions will be provided to you.

We will also contact you by phone shortly after enrolment and at 8 and 10 months to ask about the general health of your child, intake of the study formula, to check for signs of atopic dermatitis and, if necessary, go through the questionnaires on severity of atopic dermatitis. Further telephone calls are planned at the age of 18, 36 and 48 months.

You can find further information about the study on our homepage at "www.giraffe-study.com". A description of the study is also available under "www.clinicaltrials.gov/ct2/show/NCT04599946".

The study formulas are manufactured in New Zealand by Dairy Goat Cooperative, which has been producing infant formula for Europe and other parts of the world for more than 30 years. The formulas comply with European directives. Both formulas have the same nutritional composition in terms of total contents of energy, protein, carbohydrates and fat. Both formulas are available as infant milk and follow-on milk. Follow-on formula may be used from the age of 6 months onwards or after the start of complementary feeding (feeding of solids). The follow-on milk has the same energy and macro-nutrient content as infant milk, but vitamins and trace elements are adapted to the advanced age and the concurrent intake of complementary foods.

Child's food and drinks

There are no restrictions on food choices for your child. Just follow the advice of your family doctor and national nutritional recommendations. In general, you should start complementary feeding not before the age of 4 months (17 weeks) and not later than at the age of 6 months. When to start complementary feeding depends on your child's development and differs from child to child. As a guideline you can try to start if your

child can sit upright and hold his head up straight, has the oral motor skills to handle solid foods (no direct pushing out of food with tongue), and is interested in beginning and continuing to eat solids.

If possible do not feed other formula or milk than the provided study formula in the first year of life. Please use the study formula also for other foods usually prepared with milk. This will help to guarantee the success of the study.

Benefits and risks when participating in the study

By participating in this study, your child will have the opportunity to consume high-quality formula milk which has been shown to be safe and well tolerated. In addition, we will provide detailed surveillance of your child's growth, development and health and offer additional advice to you on child care and nutrition. With all infant formulas, a few infants develop intolerances. If in doubt, you can ask study team or your paediatrician or family doctor for advice. Besides the free formula, the provision of the blood count, and small gifts for your child when participating in the study visits, there are no other direct benefits by participating in the study. We will reimburse your travel costs for participating in study visits. Your participation will help to improve infant nutrition for future generations.

The risk of blood collection is negligible. It is possible that a local bruise may form and, in very rare cases, infection and inflammation at the puncture site is possible. For the stool samples, there is a minimal risk of contamination when not appropriately using tubes and storage packs.

If important new findings become known during the course of the study that could affect your decision to continue participating in this study, you will be informed immediately. You may then receive a new parental information and consent to sign if you wish to continue participating in the study. In rare cases it may be required to exclude your child from participation in the study for medical or organizational reasons. In this case, we will inform you, delete all personal contact data and use the study health data collected so far without your personal details (see also below).

Laboratory tests

Blood values provide important information to assess the effects of diet on the body. Laboratory analyses include the full blood count, however most blood results are not intended for the individual use as done in the case of illnesses by your paediatrician. The other blood analyses in the study are performed for scientific evaluations only,

and most are determined in a central laboratory with a longer time interval after blood collection. As many children as possible should participate in the blood collection, so that a sufficient number of samples can be obtained to gain meaningful insights, e.g. in relation to the development of allergies! Therefore, we very much hope that you will agree to a blood sample to be taken from your child. The blood samples are used, in addition to health tests (blood count), to measure substances related to allergies (e.g. immunoglobulins, inflammation) or different nutritional and metabolic effects of the formulas (e.g. lipidome, metabolome) that might be related to health, and genetic markers that influence the development of eczema and allergies. We will inform you about the blood count.

The stool samples are used to assess the development of healthy gut bacteria.

All samples are given a code instead of your child's name. This code is a combination of letters and numbers. The code can be related to all other study health data of your child to facilitate the scientific analysis. The code cannot be directly related to your child and ensures personal contact data protection (see below).

Genetic studies

The causes of atopic dermatitis are manifold. Genetics (inheritance) also plays a major role. Studies have shown that the skin protein Filaggrin plays an important role in the barrier function of the skin. Several changes (loss-of-function mutations) in the Filaggrin gene have been identified in patients with atopic dermatitis and are risk factors for atopic dermatitis.

If you agree to the test of the Filaggrin gene, no additional blood sample needs to be taken. The genetic material (DNA) will be extracted from the blood cells, which are left over from the blood sample taken for the other laboratory tests.

However, the Filaggrin gene is not the only risk factor for a child to develop atopic dermatitis or other allergies. Many other genetic and epigenetic factors are involved. The knowledge is constantly increasing. Until recently it was believed that genetic factors, i.e. genes, were simply present or not present, today we know much more about how genes can be "switched on and off". By examining the whole genetic material in the blood (genome-wide genotyping), we can determine which genetic variants may be relevant for the development of atopic dermatitis, related diseases and the metabolism. Furthermore, switching on or off of specific genes is of relevance can be studied (epigenetic investigation). As we are recruiting a very large number of infants in this study, which is a unique and rare opportunity for scientific

advancement, we also would like to take the opportunity to collect material for these analyses.

If you agree to the examination of the whole genetic material, the genetic material (DNA) is obtained from the blood samples of your child and examined. As for the Filaggrin gene test, no additional blood sample needs to be taken, but DNA would be collected at the ages of 4, 12 and 60 months to detect changes in gene expression.

These genetic tests will only be carried out at a later date when samples are available from as many study participants as possible. The examinations of the hereditary factors are carried out at an external institute under the auspices of the key principal investigator (Prof. B. Koletzko, LMU). Double coding (a continuous laboratory number is assigned to the coded samples before processing) prevents the employees of the external institute from drawing conclusions about personal contact data of study participants. This ensures that this particularly sensitive genetic data is additionally protected. Genetic studies are carried out for research purposes only. It is not possible and not intended to communicate results. The statistical analysis of the genetic data is carried out under the responsibility of Prof. B. Koletzko, without reference to the name of your child.

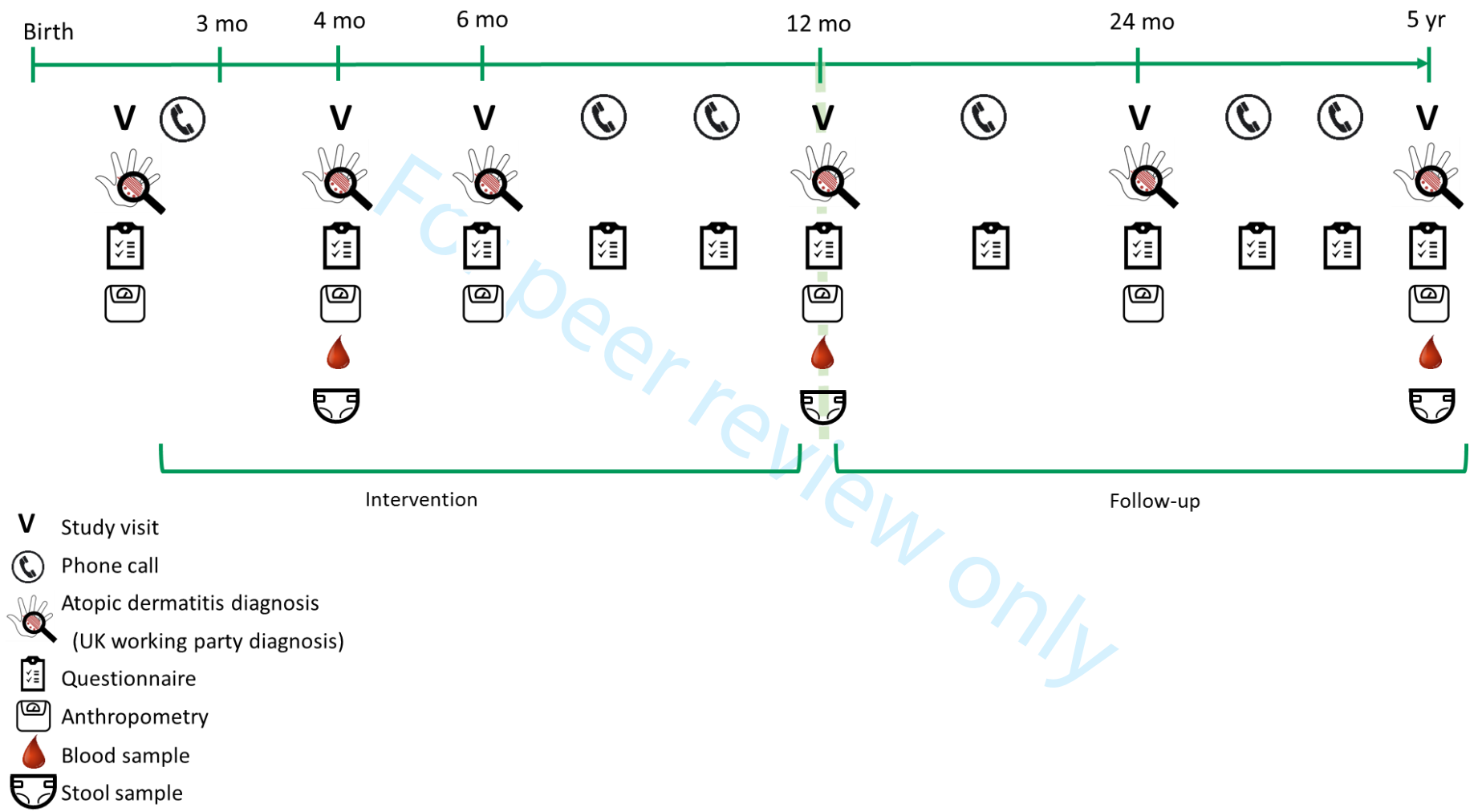
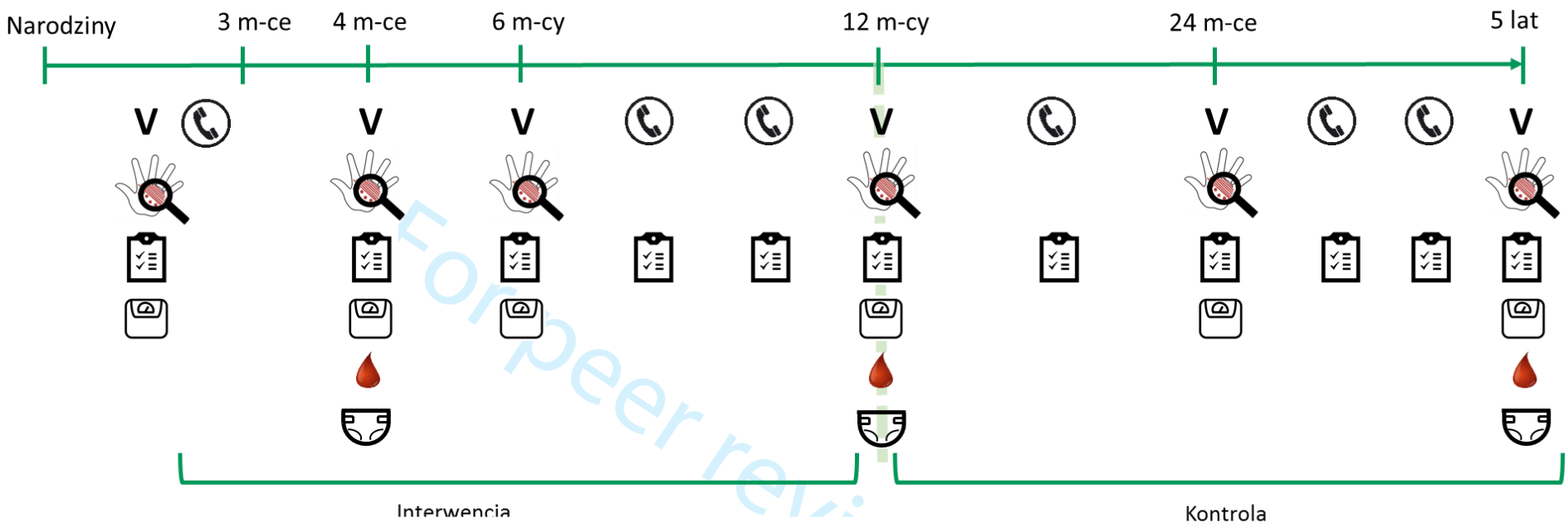


Figure 1. Study plan



V Wizyta w ramach badania

Rozmowa telefoniczna

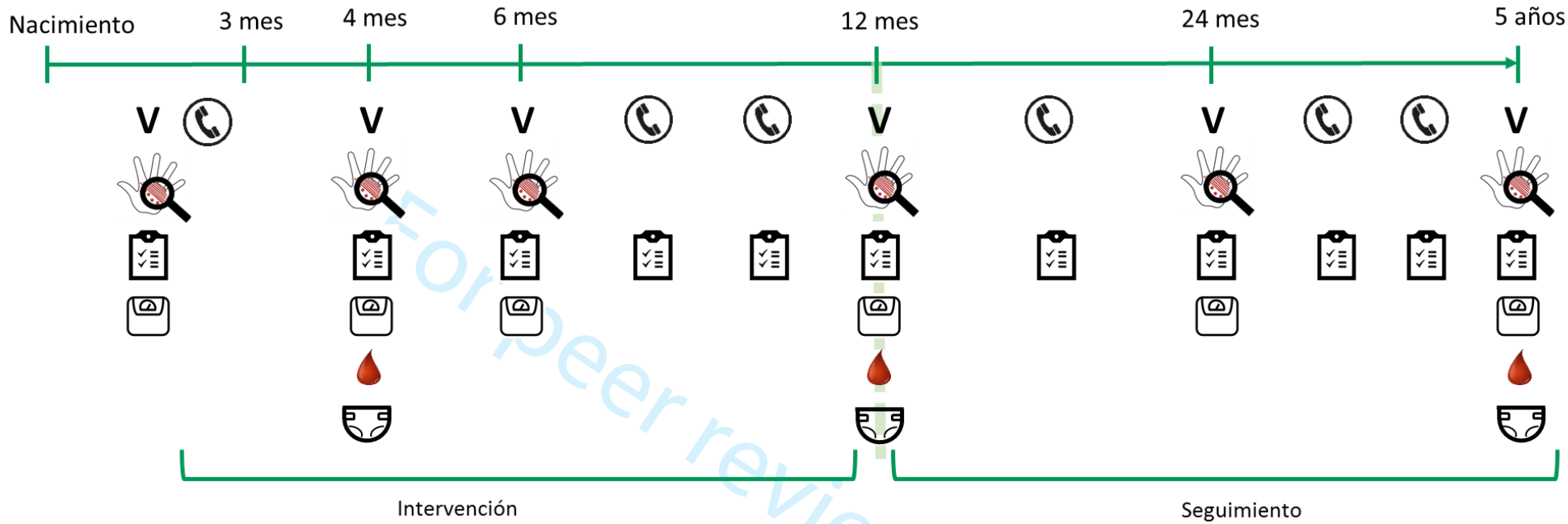
Diagnoza AZS (diagnoza grupy roboczej UK)

Kwestionariusz

Badania antropometryczne

Próbka krwi

Próbka kału



V Visita de estudio

Llamada telefónica

Diagnóstico de dermatitis atópica (DA) (Diagnóstico *UK working party*)

Cuestionario

Antropometría

Muestra de sangre

Muestra de heces

Study evaluation

The data and samples are used exclusively for scientific purposes. The study evaluation is carried out by Prof. Koletzko and his co-workers at the Children's Hospital of the University of Munich, Germany. The data interpretation and publication of study results is carried out by the scientists and medical doctors involved in the study.

Data collected in this study may be used for joint analyses with other studies (meta-analyses), which may include sharing of data with third parties. Double coding of the data as in the genetic analysis described above prevents the employees of the external institute from drawing conclusions about personal contact data of study participants.

After the last follow-up time point of the last subject, we will keep blood samples for up to 5 years to perform all planned analysis. As new insights are constantly obtained in research, we ask you to allow us to keep any excess blood samples during this period, so that blood is not wasted and is still available for possible future, innovative analyses in the context of the study.

In case any excess biomaterials (blood and/or stool) are available after this 5 year period, these will be transferred to a registered biobank (Hauner Biobank, Dr. von Hauner Children's Hospital, LMU, Germany) if you approve. Before transfer to the biobank all data that directly identify you (personal contact data) will be deleted. Your donated biomaterials and the study health data will be made available exclusively for medical research purposes. In order to realize the largest possible public benefit they can be used for a wide range of medical research. The biomaterials and the study health data are intended to be stored and made available for medical research for an undetermined period of time.

All use of data that goes beyond the context of the study will be approved by the ethical committee of the evaluation site.

Study funding

The study is sponsored by Dairy Goat Co-operative (N.Z.) Ltd (18 Gallagher Drive, Hamilton, New Zealand; www.dgc.co.nz; DGC) owned by the farmers who supply goat milk. DGC and the New Zealand Ministry for Primary Industries funded this work as part of the Caprine Innovations NZ (CAPRINZ) Sustainable Food & Fibre Futures Partnership programme. Funding covers the necessary study staff and equipment, all planned aspects of the study, laboratory tests and provision of study formula. Any future

scientific investigations will be carried out with further industrial funding or state support.

Insurance

Although no complications are expected, all study participants are covered by a study insurance. The insurance covers all damage to health that occurs as a result of the measures applied in connection with the study up to a maximum amount of € XXXX.

In case of damage, you can contact the insurer directly (xxx, tel.: xxx; policy number: xxx) and assert your claims. To ensure the insurance cover is not jeopardized, you must tell us all medical treatments that your child undergoes during the study phase (exceptions are preventive examinations and vaccinations). This also applies to the use of new medications. If you or your child have any damage to your health that may have occurred as a result of participating in the study, please inform the relevant study staff and the above-mentioned insurance company.

Voluntariness / Withdrawal Clause

Participation in the study is voluntary. With your signature on the "Consent Declaration" you give your consent to your child's participation in this study. You have the right to stop participating in the study at any time without giving reasons and without disadvantages.

Compensation

For participation in the study you will be compensated for expenses.

If you have further questions about this study or if you think you or your child have suffered a study-related health impairment, we are at your disposal; Tel.: xxx E-mail: GIraFFE.Studie@xxx.xx

Data protection:

The following data protection rules apply as part of the study.

Data protection: This study complies with the rules on medical confidentiality and data protection in accordance with the European and [your countries] directives and the Helsinki Declaration. Your contact details will be stored in a database (MedSciNet, Stockholm, Sweden, http://medscinet.com/). This database only stores personal contact data, but no medical data. In order to deliver the study formula, your contact details are passed on to an external logistics company (xxx). The company is prohibited from using this data for purposes other than the delivery of the study formula. The company is subject to [XXX] statutory data protection regulations.

All other data - i.e. "study health data" - which are not used for contact organisation are stored in separate database (MedSciNet, Stockholm, Sweden, http://medscinet.com/ as well as in the hospital of the University of Munich). Personal contact data such as name or address is not collected in this database. The assignment to your child's name can only be done using a code, which can only be assigned to a name with the active help of the staff at the study center. Thus, all collected data and findings of your child are pseudonymized.

You have the right to receive information about your stored personal contact data at any time, to correct it or, if necessary, to have it deleted.

Responsible for data processing is Prof. [local PI].

Contact details of the data protection officers:

In the event of a complaint, you have the right to contact the respective data protection supervisory authority. For [your institution] this is:

Data Protection Officer

[XXX contact information of local data protection officer]

The higher authority for [your institution] is:

[contact information of a federal or similar higher level data protection officer]

Data access:

Access to personal database with name, contact details, contact information, and ID codes (connecting study health data to personal contact data) is limited to persons involved in the study under supervision of Prof. [local PI]. For organizational reasons and to monitor the study, also personal under the supervision of Prof. B. Koletzko (LMU) will have access to the personal database. Dairy Goat Co-operative Ltd. can commission monitoring of the quality of the study. The monitor (currently Uta Clausen) is committed to data protection and has access to personal and study health data on site. Decoding of individual study participants is only carried out for safety reasons ("medical reasons"). The monitoring company is subject to the local, statutory data protection regulations. All persons with access to the data are listed in a log file and have a personal, traceable login.

Dairy Goat Co-operative Ltd, LMU and the study centres/sites have access to study health data. Dairy Goat Co-operative Ltd. has never access to personal contact data. Study centers only have access to the personal contact data of participants at their site(s). Use of a code will protect your identity and ensure the confidentiality of your data. As data controller, LMU will apply contractual, organizational and security measures ensuring the maintenance of an adequate protection level required by the European and [study site's country] statutory data protection regulations. During those procedures, you and your child identity will not be disclosed.

For the laboratory analyses, the blood samples are only passed on with a code and do not allow any conclusions to be drawn about an individual study participant. The storage of the samples and some laboratory analyses are carried out in laboratories of the hospital of the University of Munich. Genetic analyses and some further examinations are carried out at external institutes. For the genetic analyses, a 2nd encryption by the employees of the external institute is carried out. This double coding ensures that the genetic data is additionally protected. Unblinding is only possible through the study center, but not through the external institute.

In case of withdrawal of consent, the name and your personal contact details will be deleted from our database. Your child's data stored until then will now be used anonymously. In addition, the name and personal contact details of all study participants will be deleted within one month of completion of the study (including analysis of bio-samples). The written documents, including this declaration of consent, will be kept in the study center until the end of the study and in a suitable warehouse until the end of the statutory retention period (12 years after the end of the study).

In the case of publication of the study results, the confidentiality of your child's personal contact data is also guaranteed, as the data is reproduced, if at all, in an anonymized form. On request, we will inform you about general study results. In the event of additional investigations or data collection that go beyond the above-mentioned course of study, we will

Before you enter the study, you have the opportunity to write down specific questions, which should be discussed in more detail with you.

Consent & Privacy Policy for the participation of my/our child in the GIraFFE study

Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis

(GIraFFE study)

Surname, first name of the child	Birth date

The study conditions have been fully explained to me and all questions have been clarified to my satisfaction. I have received the form with the study information. I have had plenty of time to read this form and ask questions. Possible risks and disadvantages for my child were explained to me. I know that I can ask any question about this study and the investigations now and in the future. I know that I/my child can withdraw from the study at any time without having to give reasons or that I or my child would suffer any disadvantages. I hereby consent to my child's participation in the study:

Date	Surname, First name 1. parent or legal guardian	Signature 1. parent or legal guardian

I have sole custody: ☐ Yes ☐ No

Date	Name, Forename 2. parent or legal guardian	Signature 2. parent or legal guardian

Date	Name, Forename Study personnel	Signature

I have taken note of the data protection information within the scope of the participant information. I hereby consent to the collection and use of my child's personal contact data in accordance with these conditions.

Date	Surname, First name 1. parent or legal guardian	Signature 1. parent or legal guardian

Date	Name, Forename 2. parent or legal guardian	Signature 2. parent or legal guardian
Date	Name, Forename Study personnel	Signature Study personnel

Consent & Privacy Policy for the genetic examination of my/our child in the GIraFFE study and for biobanking

Effects of infant feeding with goat milk formula or cow milk formula on atopic dermatitis

(GIraFFE study)

Surname, First name of the child	Birth date

☐ I hereby agree that genetic material may be extracted, stored and examined from my child's blood. Genotyping in the GIraFFE study is used to identify a possible genetic cause of a **modified skin protein (filaggrin)** that may be related to the appearance of atopic dermatitis (eczema). Participation in the examination does not involve any further health risks beyond the blood collection.

☐ I hereby agree that genetic material may be extracted, stored and examined from my child's blood. The **genome-wide genotyping and epigenetic investigations** serve to uncover the genetic causes of diseases and causes of allergies and metabolic changes within the GIraFFE study. Participation in the examination does not involve any further health risks beyond the blood collection.

☐ I hereby agree that any excess bio-samples are transferred together with anonymized study health data to a registered biobank as described in the study information.

The data and results will be used exclusively as outlined in the subject information. Only authorized employees of the study can access the encrypted data. Data will not be passed on to unauthorized third parties. The genetic data obtained in the course of this study shall be retained for up to 10 years after completion of the scientific study or until revocation has been made.

I know that I can ask further questions now and in the future about this study. I know that I can withdraw from voluntary participation in the study at any time without having to give reasons. I voluntarily consent to the collection, processing and use of personal contact data in accordance with the information sheet of the study.

Date	Surname, First name 1. parent or legal guardian	Signature 1. parent or legal guardian

Date	Surname, First name 2. parent or legal guardian	Signature 2. parent or legal guardian
Date	Surname, First name Study personnel	Signature Study personnel

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	✓ various pages
Protocol version	#3	Date and version identifier	13
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 2, 14, 15
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1, 2
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team,	14

		and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3, 4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3,4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data	1, 2, 5

		will be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5, 6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6, 7, suppl. material
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a no
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical	7, 8

		relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9, 10,11
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and	9, 10, 11

		validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7, 9, 10
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a not planned
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	13

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	13
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12

Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a not yet decided
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a not yet planned
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental Material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a