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Multicentre randomized controlled trial of protein content in toddler formula during the second year of life: Protocol of the ToMI trial

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Abstract

Introduction Reduction of milk protein content in infant formula provided during the first year of life has been shown to reduce early weight gain and obesity later in life. While rapid weight gain during the first two years of life is one of the strongest early predictors of obesity, the role of animal protein intake beyond the first year of life is unclear. The aim of this study is to examine the role of milk protein during the second year of life in healthy children on weight gain and obesity risk in preschool age.

Methods and analysis This randomized, double-blinded study enrolled 1,618 children aged 11.5 to 13.5 months in Spain and Germany into 2 groups receiving isocaloric toddler milk with differing protein content during the second year of life. The experimental formula contains 1.5g/100kcal and the control formula 6.15g/100kcal protein and otherwise equal formula composition, except for modified fat content to achieve equal energy density. The primary endpoint is BMI-for-age z-score at the age of 24 months. The children are followed until 6 years of age.

Ethics and dissemination Ethics approval was obtained from the ethical committees of the LMU University Hospital Munich, Germany (Nr. 555-15) and at Institut d'Investigació Sanitaria Pere Virgili, Reus, Spain (Ref. CEIm IISPV 013/2016). We aim at publishing results in peer-reviewed journals and sharing of results with study participants.

Trial registration number NCT02907502

Strengths and limitations of this study

- This study uses a randomized and double blinded design to minimize potential confounding and biases.
- The multicentre design of this study with sites in Spain and Germany increases external validity of study results.
- The follow-up of the cohort is planned until six years of age and will provide the possibility to examine long-term effects of the intervention.
- Conclusions will be limited to effects of dairy protein provided with milk based drinks in the second year of life and cannot be extrapolated to effects of total dietary protein supply.

Keywords

Toddler milk; milk protein; protein intake; clinical trial; obesity; BMI

Introduction

A randomized double blind controlled clinical trial demonstrated that reducing protein intake in infant formula provided in the first year of life lowers early weight gain until 2 years of age [1]. Data from the same study (CHildhood Obesity Project [CHOP] trial) demonstrated that lower protein supply with formula fed in the first year of life also reduced BMI and obesity risk at school age [2]. The results of the CHOP trial contributed to enhanced promotion of breastfeeding and efforts in reducing the protein content in infant and follow-on formula [5 6].

It remains unclear which child age period is most sensitive to a modified protein intake, and whether limiting protein intake during the second year of life would also achieve benefits for prevention of excessive weight gain and later obesity. Observational studies find a consistent association of later overweight and obesity with total protein intake and in particular of milk protein intake, not only during infancy but also during the preschool age [7-11]. A systematic review on the effects of dietary protein intake concluded that the first 2 years of life are the most sensitive time period [12].

The untoward programming effect of a high early protein intake on later obesity risk has been linked to its effects on increasing plasma and tissue concentrations of insulinogenic amino acids, insulin and insulin-like growth factor 1 (IGF-1), which appear to induce a higher weight gain during the first 2 years of life as well as an enhanced adipogenic activity [13]. Such effects of an infant formula higher protein content on insulinogenic amino acids, insulin and IGF-1 levels have been shown in the double-blind randomized CHOP trial [15-17].

Milk protein seems to play a key role in growth regulation during early childhood. Protein intake is the main contributor for nutritional regulation of the IGF-I axis [19 20]. Milk protein enhances serum IGF-1 to a greater extent than meat protein [21]. This might explain the more pronounced effect of milk protein compared to other proteins on the later risk of obesity that has been reported [10].

Average protein intake of young children in Europe and other regions is much higher than metabolic requirements. During the second year of life, 30-50% of total daily protein is comprised of dairy products [25 26], indicating particular opportunities to reduce overall protein consumption though modifying dairy protein intake.

Therefore, we designed a randomized controlled trial to examine the role of milk protein intake during the second year of life on child growth and later obesity risk. If a reduction of milk protein during the second year of life has an appreciable effect on growth and obesity development, respective dietary modification may be translated into the practice of toddler feeding.

Main Objective

We aim at evaluating the effect of two iso-energetic milk products for young children with differing protein content on growth during the second year of life.

Methods and analysis

Study design and population

The Toddler Milk Intervention trial (ToMI trial) is designed as a two-arm, parallel, randomized, double blind controlled trial to evaluate toddler milk products with different protein content. The study is conducted at university hospitals in Munich, Germany, and in Tarragona and Reus, Spain.

The target population are healthy children at the age of one year. The children are enrolled if they meet the inclusion and exclusion criteria outlined in Table1.

Intervention

Formula composition

Two investigational formulas are used. The experimental formula contains 0.72g protein/100ml (1.5g/100 kcal), with a protein content that is similar to breast milk in advanced lactation. The control formula contains 2.95g protein/100ml (6.15g/100 kcal) which is comparable to standard cows´ milk. Contents of energy, carbohydrates, vitamins and minerals are very similar for both formulas (Table 2). In order to reach the same energy content in both formulas, the fat content varies between experimental (4.25g fat/100 kcal) and control formula (2.16g fat/100 kcal) but the lipid composition and the ratio of milk fat/vegetable oils is the same.

Dose, route of administration and schedule of formula

Participating families receive the formula as milk powder (one can comprises about 400g of product) and are advised to prepare the formula according to the instructions. It is recommended to consume at least 300ml of formula per day. Further, parents are encouraged to substitute with the study formula any milk intake from the child´s diet. The intake of other dairy products such as cheese or yoghurt is accepted.

The intervention starts with the first study visit at around one year of age and ends with the third study visit at around two years of age. The study formula is given to the parents at no costs and is delivered directly to subject's home. Subject's compliance is regularly checked by telephone and personal interviews. After the end of the intervention, return and pick-up of remaining cans is organized. If not possible, families are advised to destroy remaining infant formula cans.

Discontinuation criteria

Discontinuation of the trial can be either due to withdrawal of consent at any time or due to the investigator´s decision that continuation within the trial might impair child´s health.

Outcome measurements

Primary endpoint

The primary endpoint is BMI-for-age z-score (based on the WHO Multicentre Growth Reference Study [27]) at the age of 24 months.

Secondary objectives and endpoints

The secondary objectives serve to evaluate the safety and efficacy of the two milk products used and to complement the primary endpoint. Secondary endpoints are:

- BMI-for-age z-score at 72 months,
- The percentage of overweight and obese children at 24 months of age according to CDC definition: Overweight is at and above the 85th to less than 95th percentile and obese 95th percentile or greater,
- The percentage of overweight and obese children at 72 months of age,
- Anthropometric measures (z-scores for weight, length and head, waist and arm circumference at 12, 18, 24, 48 and 72 months of age; hip circumference at 48 and 72 month of age),
- Subcutaneous fat distribution (from skinfold thickness at 12, 18, 24, 48 and 72 months of age),
- Total body fat and lean mass (from BodPod measurements at 24, 48 and 72 month of age),
- Blood pressure (48 and 72 month of age),
- Child development (24 and 48 months of age),
- Metabolic and endocrine markers (IGF-1, IGF-BP2, IGF-BP3, insulin, leptin, adiponectin, ghrelin, lipid profile and complete blood count at 12, 24 and 72 month of age),
- Serum albumin, urea, creatinine, amino acids at 12, 24, 72 months of age and ferritin and 25-OH-vitamin D (at 24 months of age),
- Metabolic profile (from plasma at 12, 24 and 72 months of age and from urine samples at 12, 18, 24, 48 and 72 months of age),
- Urine markers (Calcium, C-peptide, creatinine urea nitrogen at 12, 18, 24, 48 and 72 months of age),

Furthermore, the following hypotheses will be examined:

- Total energy intake is not affected by the low protein formula.
- Total protein intake is lower in the group of protein reduced formula.

- Plasma concentrations of essential amino acids and of IGF-1 at the age of 24 months are lower in the low protein formula group compared to the high protein formula group.
- Systolic and diastolic blood pressure measurements at the ages of 48 and 72 months are lower in the low protein formula group compared to the high protein formula group.
- Body fat mass at age 24 months is lower in the low protein formula group compared to the high protein formula group.
- DNA methylation affects the association of protein intake and BMI
- Protein intake affects DNA methylation
- DNA methylation affects the association of protein intake and the metabolic profile

Sample size

The sample size calculation is based on the observations from the CHOP-study [1]. This trial examined the difference in BMI-for-age z-scores between two groups of children fed a higher or lower formula during the first year of life. At 24 months of age the BMI for age z-score difference between both formula groups was 0.2. The absolute difference in protein content between intervention and control group in the CHOP-trial was lower (Infant formula: 0.8g/100ml; Follow-on formula: 1.6g/100ml) compared to the ToMI-trial (2.2g/100ml). Despite a higher protein difference, we expect a lower effect of the intervention due to the lower contribution of milk to the total protein intake in the second year of life. Thus, we assume a slightly lower mean difference in BMI for age z-score of 0.15 at 24 months of life.

The sample size was calculated with the BMI for age z-score of 0.15 and a standard deviation (sd) of 0.9. Assuming a power of 80 % and a significance level of 5% (two-sided alpha of 0.05), a sample size of 566 subjects per intervention arm is calculated. Therefore, 1,132 subjects in total are needed. To have enough power to detect also a difference at 72 months (6 years) of age, at an assumed loss to follow-up of 30%, a final sample size of 1,618 subjects was estimated.

Recruitment

The study sites in Munich, Reus and Tarragona followed somewhat different recruitment strategies due to different local conditions. In Germany all inhabitants are registered in central registries. The public registries provided the study team for this defined research on a regular basis addresses of all families with children in the required age group (about 26,000 per year). These families living in Munich and about 70 surrounding municipalities were contacted once by postal mail and invited to contact the study team if interested in participation in the trial.

In Spain two recruitment strategies were used for both sites covering about 3000 births per year. First, telephone contacts from families who delivered their child at either of the two hospitals were available. These families were contacted directly. Second, recruitment interviews at primary health

care centers were conducted. In these primary health care centers, Spanish children are seen for health care examinations and for vaccinations.

Allocation of study formula and blinding

The study formula cans are labelled with one of eight codes. Four codes each are assigned to the intervention or the control group, respectively. The allocation of the codes is performed online by study staff after check of in- and exclusion criteria within the data capture tool (iMedidata, Medidata Balance, New York, USA) using balanced randomization stratified by country. After enrolment of the subject into the trial, study staff dispense the assigned study formula to the study participant along with instructions for formula preparation.

The study is double blinded with all persons involved in local organization and conduct of the study such as study staff, principal investigator, project manager, biostatistician, data manager, trial monitor and laboratory analysts being unaware of the code allocation. After the code break for the primary outcome analysis, subjects will receive a new identification id in the analysis data to hamper the unblinding for above persons in the further follow-up. An emergency code break by an Investigator may be requested only in case of an unexpected serious adverse event (SAE) suspected to be related to the investigational product.

Data collection, management and analysis

Data collection and management

During the intervention period three visits at the hospital are scheduled at 12, 18 and 24 months of age (Figure 1). At baseline socioeconomic data and data on health, growth and nutrition during the first year of life are assessed. At each visit anthropometric measurements are performed, urine samples and dietary intake records are collected. Blood is taken at 12 and 24 months of age. Additionally, at 24 months of age body composition using an air displacement plethysmography (BodPod COSMED, Rome, Italy) as well as physical activity measurement using an accelerometer device (Actigraph wGT3X-BT, Pensacola, FL, USA) is performed. Further, data of child´s development based on parent answers of the Ages & Stages questionnaire (ASQ-3, Brookes Publishing Co., Inc., USA) are collected.

For follow-up, two additional visits are scheduled at 48 and 72 months of age with anthropometric, body composition and physical activity measurements and collection of urine samples and food frequency questionnaires. At 48 months of age, the ASQ-3 is used again. Blood is taken at 72 months of age.

During all study visits and at several additional telephone calls between visits, parents are asked for health problems (including adverse events) and compliance. For compliance the intake of study milk

and any discontinuation of study milk intake with reasons are determined. The number of consumed cans will be used to determine the average study milk consumption.

Collected data is organized in different databases. To organize and document all contacts with study participants and to coordinate the shipment of the study product, a web-based participant management tool is used (developed jointly with MedSciNet AB, Stockholm, Sweden). In this database, personal data is saved and stored on a secured data server. This database is separated from the other databases which store all medical, nutritional and laboratory data.

All collected health data are primarily captured on paper except data from questionnaires on physical activity and food frequency questionnaires based on the Idefics study that are partly entered by families using LimeSurvey (LimeSurvey GmbH, Hamburg, Germany). All other health data is entered into a further web-based database (iMedidata, New York, USA). Nutritional data from 24-hours recalls are entered into Nutritics (NUTRITICS LTD, Dublin, Ireland) with nutritional information from the German nutritional database BLS 3.02 and complemented with the nutritional composition from a variety of commercial infant foods and local foods, obtained directly from the label, producer websites or local food composition databases.

Laboratory samples are processed according to a laboratory SOP. In general, aliquots have 2D barcodes, are scanned, linked with the subject ID and stored into 96-well racks at -80°C for later analysis. Only blood count, lipid status and HbA1c are measured locally on the day of blood sampling.

To ensure data quality, study staff is trained in regular intervals, and procedures are harmonized among study centers by regular contact and monitoring. Furthermore, anthropometric measurements are performed at least twice and data entry is strictly checked for consistency and plausibility by the monitor. Standard operating procedures for all measurements are in place; anthropometric measurements are based on the WHO Growth Standards study [27].

Statistical methods

A statistical analysis plan is created before final code break for the analysis of primary and secondary outcomes. For the statistical analysis, the full analysis dataset (FAS) and the per-protocol-dataset (PP) will be considered. The FAS comprises all randomized subjects who consumed at least one can of investigational product. The PP compromises all subjects included in the FAS with a mean consumption of the recommended daily minimum amount of investigational product (300ml/d). No imputation of missing values is foreseen.

The primary endpoint will be analyzed by linear regression (ANCOVA) and corrected for BMI-for-age z-score at baseline, study center and gender. The results of the final model will be compared to further adjusted models; possible effect modification of the primary outcome will be also considered.

Secondary analyses supporting primary objective:

1. BMI-for-age z-score at 72 months.
2. The percentage of overweight and obese children at 24 months of age according to CDC definition: Overweight at and above the 85th to less than 95th percentile and obese 95th percentile or greater.
3. The percentage of overweight and obese children at 72 months of age.

In order to control the experiment wise false positive rate, the listed hierarchy (primary – secondary endpoints) will be maintained in interpreting these outcomes. The incidence of overweight and obese children at 24 and 72 months of age shall be also estimated according to International Obesity Task Force IOTF definition [30]. The percentage of overweight and obese children will be analyzed by the method of O. Sauzet, et al. [31].

Secondary endpoints include anthropometric measures, dietary and biochemical data. We will use z-scores of WHO growth standards for anthropometry measures at months 12, 18, 24, and 48. We will use a likelihood-ratio test to examine if there is a longitudinal treatment effect. Additionally, treatment differences at each visit will be analyzed using ANCOVA. The ANCOVA approach was chosen so that treatment differences and p-value do not depend on the stage of analysis. A further supportive analysis with a mixed linear model shall be performed at stage 3. Fixed effects shall be the intervention group, age, gender, and age times intervention group. The random effects shall be a random intercept and slope.

Dietary data is collected by 24-hours recalls or food frequency questionnaires, which allow us to test for differences in macronutrient intake using ANCOVA. Hence, we are able to analyze if subjects change their dietary habits over time.

Biochemical data is often log-normal distributed. In order to analyze this kind of data properly, we will log-transform the data to achieve approximately normal distributed residuals.

Monitoring

Data monitoring

To ensure safety of the intervention, an interim analysis is planned when 260 subjects have completed the intervention (at 24 months of age). Non-inferiority for growth has to be shown. If this is the case, the study is continued as planned. Otherwise, a second stage interim analysis is performed including the first 390 subjects who have completed the intervention. Non-inferiority is shown when in FAS as well as in PP the lower bound of the two-sided 95% confidence interval of the treatment difference (estimated model) is larger than the non-inferiority margin. Furthermore, the safety evaluation will

consider endpoints including adverse events, anthropometry, laboratory data and protein intake. Based on the results of the interim analysis and in accordance with the charter of the Data Monitoring Committee, the DMC will recommend either continuing the study as planned or performing the second stage interim analysis. The DMC is independent and consists of expert clinicians and statisticians with no competing interest. The planned interim safety analysis took place in June 2018 and no safety concerns were detected.

Besides the interim analysis, safety is continuously observed by blinded online monitoring of individual growth curves based on the WHO growth charts. If a considerable number of subjects drop below the median growth curve, an interim analysis will be initiated and the DMC will review unblinded data.

Harms

Any adverse events (AE) which lead to an untoward medical occurrence except for diagnostic and therapeutic non-invasive and invasive procedures will be recorded during the entire intervention period until 30 days after last study milk intake. After these 30 days, only AE´s which are related to the intervention treatment will be recorded. Each AE will be rated according to its severity and its relationship to the study milk. Additionally, severe adverse events (SAE) which e.g. requires inpatient hospitalization will be reported to the safety manager within 24 hours after notice and will be followed up until the outcome is known. A participant insurance is in place.

Monitoring

A commercial monitoring company reviews the process, AE reporting, data capturing and corresponding source data on a regular basis to ensure protocol compliance, accuracy and completeness.

Protocol versions

Issue date: 15.09.2020; version identifier: 5; number of protocol amendments: 5; initial version: 9 March 2016. First modification: 30 March 2016. Besides adaptation from requests of both ethical committees before the start of the study and several minor changes due to misspecifications in the protocol, several clarifications were needed, e.g. to provide more clarity and criteria for study termination before regular completion of the study, clarification in the statistical interpretation of secondary endpoints, addition of new secondary endpoints physical activity and HbA1c, the adaptation to the new European data protection rules in 2018, and a change in exclusion criteria to allow the inclusion of children that are breastfed once per day. Furthermore, an extensive specification of the safety interim analysis after inclusion of 260 children was added in 2018 and more details for collection of AEs separating the collection into two periods, during and after the intervention, were provided.

Ethics and dissemination

Ethical considerations

This study is conducted in compliance with the International Conference on Harmonization (ICH) guidelines and the Declaration of Helsinki and complies with Good Clinical Practice guidelines. Ethics approval was obtained from the ethical committees of the university hospitals at the Ludwig-Maximilian University in Munich, Germany (Projekt Nr. 555-15) and at the Institut d'Investigació Sanitaria Pere Virgili, Reus, Spain (Ref. CEIm IISPV 013/2016. All protocol amendments were and will be approved by the ethical committee prior to implementation.

Written informed consent is collected by study staff from all legal guardians prior to study inclusion in adherence with regulatory requirements. Each subject receives oral as well as written informed consent in plain language with adequate time in advance to make an informed decision about study participation. The informed consent form for both study sites is enclosed in the online supplementary.

Patient and Public Involvement

The study protocol was primarily developed at a public university hospital without involvement of the sponsor. There was no further public or patient involvement.

Public dissemination and data availability

Study results will be published in peer-reviewed journals and presented on national and international conferences. Study results will also be communicated to participants. Results will be written-up and published by the investigators without help of professional writers. Authorship will depend on relevant contribution to the study. The full study protocol will be made available upon request. The participant-level dataset is not currently planned to be available because consent was not obtained for the sharing of such data from participant's parents / legal guardians or the Institutional Ethics Committees.

Trial status and time course of the trial

The study started to recruit subjects in September 2016 and finished recruitment of 1,625 children in October 2019. The intervention phase will last until October 2020. The database closure for the analysis of the primary outcome is planned for the first quarter of 2021. The follow-up will be completed around October 2025.

Funding, role of the sponsor and investigators

The sponsor has allocated a fixed budget for each study center to recruit and follow the subjects. The sponsor is producing the study product and distributes the study product to the study subjects. The sponsor is funding the monitoring of the study. The primary protocol was outlined by the investigators and was jointly further developed by investigators and sponsor. Data management will be primarily

done by the sponsor, except parts of the compliance checks, checks of biosamples and body composition data, as well as nutritional and physical activity data. The primary analysis will be performed by the sponsor. The investigators have to approve the statistical analysis plan and will have full access to all the data. Any published interpretation of the data has to be in mutual agreement between sponsor and investigator without hampering the research freedom of the investigators. The urinary metabolic profile will be performed by the sponsor, all other laboratory measurements by the investigators. BK is the coordinating principal investigator with VG being his deputy, JE is principal investigator in Spain.

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Authors´ Statement

VG and VJ wrote the manuscript. VG and BK provided the original outline of the protocol; JE, MZ, MG, and DG participated in the design and set-up of the study. BK, JE, MZ, MG, and DG critically revised the content of the manuscript.

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Conflict of interest

The institutions of VG, VJ, BK, JE, MZ, MG receive funding by the sponsor to conduct the study- and DG is employed by the sponsor of the study.

Tables

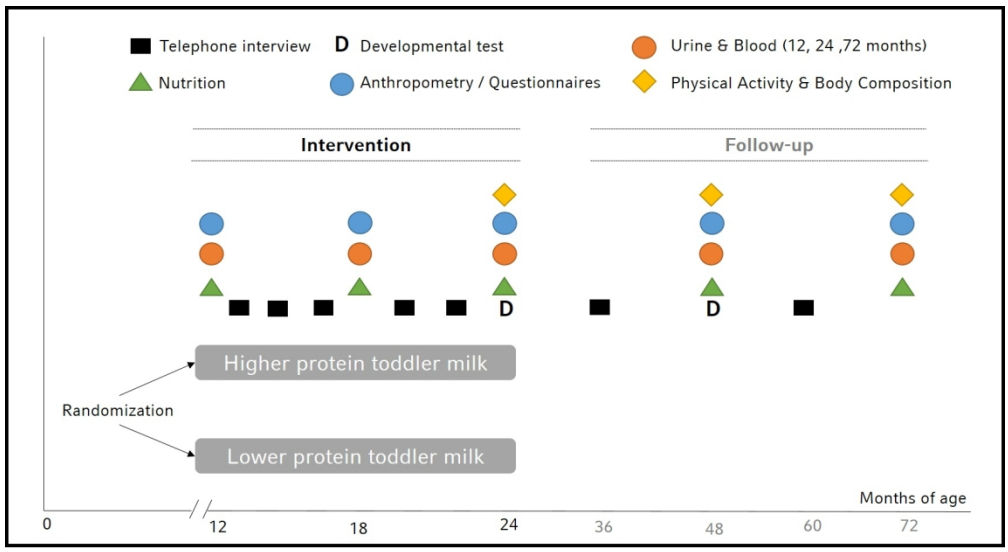
Table 1: Inclusion and Exclusion criteria of the Tomi trial

Inclusion criteria	Exclusion criteria
• Legal guardians signed the written informed consent.	• Infant who is breastfed at least twice in 24 hours at time of enrolment.
• Child was born full term (≥ 37 + 0 weeks of gestation).	• Infant who usually does not drink 300 ml of cow´s milk and/or formula milk per day.
• Child´s birth weight is between 2.5 and 4.5 kg.	• Cow´s milk allergy.
• Child is born from a singleton pregnancy.	• Lactose intolerance.
• Child´s age at enrolment is between 11.5 and 13.5 month.	• Institutionalized children.
• Child´s legal guardians are of legal age and they have sufficient local language skills to understand the study information, informed consent and study procedure.	• Diagnosed disorder, which interfere with nutrition or growth (e.g. celiac disease, inflammatory bowel disease).
• Child and child´s parents are willing to fulfil the requirements of the study protocol and procedures.	• Children who participated in any other interventional clinical trial 4 weeks prior to enrolment.
• Child´s family is available via phone or e-mail throughout the whole study.	

Table 2: Nutritional composition of the interventional products (toddler milks)

	Experimental toddler milk (ready to drink, per 100ml)	**Control toddler milk** (ready to drink, per 100ml)
Energy	201 KJ/48 kcal	201 KJ/48 kcal
Protein	0.72 g	2.95 g
Fat	2.0 g	1.0 g
Saturated fatty acids	0.8 g	0.4 g
Carbohydrates	6.7 g	6.7 g
Lactose	6.7 g	6.6 g
Other	<0.1 g	<0.1 g
Salt	0.1 g	0.1 g
Vitamines		
Vitamine A	71 µg	66 µg
Vitamine D	1.2 µg	1.3 µg
Folic acid	14.9 µg	14.2 µg
Vitamine B12	0.2 µg	0.2 µg
Vitamine C	6.4 mg	6.9 mg
Minerals		
Calcium	115 mg	115 mg
Micronutrients		
Iron	0.5 mg	0.5 mg
Zinc	0.3 mg	0.6 mg

Figure 1: Assessments in children participating in the ToMI trial



Assessments in children participating in the ToMI trial

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Check/page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Y
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	10
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 13
	5b	Name and contact information for the trial sponsor	13
	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	13
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13
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
	6b	Explanation for choice of comparators	
Objectives	7	Specific objectives or hypotheses	4
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, noninferiority, exploratory)	4

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
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)	4, Table
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4, Table
	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)	5
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
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 relevance of chosen efficacy and harm outcomes is strongly recommended	5,6,
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)	7
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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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	7

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	
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
Methods: Data collection, management, and analysis			
Data collection methods	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 validity, if known. Reference to where data collection forms can be found, if not in the protocol	7,8
	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
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	8,10
Statistical methods	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	8,9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
	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)	8
Methods: Monitoring			
Data monitoring	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	10

	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	10
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	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	10
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	11
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)	11
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	yes
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	8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
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	10
Dissemination policy	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	11
	31b	Authorship eligibility guidelines and any intended use of professional writers	11
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplement
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	No

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effect of milk protein content in toddler formula on later BMI and obesity risk: Protocol of a multicentre randomized controlled trial (ToMI)

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Effect of milk protein content in toddler formula on later BMI and obesity risk: Protocol of a multicentre randomized controlled trial (ToMI)

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Abstract

Introduction Reduction of milk protein content in infant formula provided during the first year of life has been shown to reduce early weight gain and obesity later in life. While rapid weight gain during the first two years of life is one of the strongest early predictors of obesity, the role of animal protein intake beyond the first year of life is unclear. The aim of this study is to examine the role of milk protein during the second year of life in healthy children on weight gain and obesity risk in preschool age.

Methods and analysis This randomized, double-blinded study enrolled 1,618 children aged 11.5 to 13.5 months in Spain and Germany into 2 groups receiving isocaloric toddler milk with differing protein content during the second year of life. The experimental formula contains 1.5g/100kcal and the control formula 6.15g/100kcal protein and otherwise equal formula composition, except for modified fat content to achieve equal energy density. The primary endpoint is BMI-for-age z-score at the age of 24 months adjusted for BMI at 12 months of age. The children are followed until 6 years of age.

Ethics and dissemination Ethics approval was obtained from the ethical committees of the LMU University Hospital Munich, Germany (Nr. 555-15) and at Institut d'Investigació Sanitaria Pere Virgili, Reus, Spain (Ref. CEIm IISPV 013/2016). We aim at publishing results in peer-reviewed journals and sharing of results with study participants.

Trial registration number NCT02907502

Strengths and limitations of this study

- This study uses a randomized and double blinded design to minimize potential confounding and biases.
- The multicentre design of this study with sites in Spain and Germany increases external validity of study results.
- The follow-up of the cohort is planned until six years of age and will provide the possibility to examine long-term effects of the intervention.
- Conclusions will be limited to effects of dairy protein provided with milk based drinks in the second year of life and cannot be extrapolated to effects of total dietary protein supply.

Keywords

Toddler milk; milk protein; protein intake; clinical trial; obesity; BMI

Introduction

A randomized double blind controlled clinical trial demonstrated that reducing protein intake in infant formula provided in the first year of life lowers early weight gain until 2 years of age [1]. Data from the same study (CHildhood Obesity Project [CHOP] trial) demonstrated that lower protein supply with formula fed in the first year of life also reduced BMI and obesity risk at school age [2]. The results of the CHOP trial contributed to enhanced promotion of breastfeeding and efforts in reducing the protein content in infant and follow-on formula [3 4].

It remains unclear which child age period is most sensitive to a modified protein intake, and whether limiting protein intake during the second year of life would also achieve benefits for prevention of excessive weight gain and later obesity. Observational studies find a consistent association of later overweight and obesity with total protein intake and in particular of milk protein intake, not only during infancy but also during the preschool age [5-9]. A systematic review on the effects of dietary protein intake concluded that the first 2 years of life are the most sensitive time period [10].

The untoward programming effect of a high early protein intake on later obesity risk has been linked to its effects on increasing plasma and tissue concentrations of insulinogenic amino acids, insulin and insulin-like growth factor 1 (IGF-1), which appear to induce a higher weight gain during the first 2 years of life as well as an enhanced adipogenic activity [11]. Such effects of an infant formula higher protein content on insulinogenic amino acids, insulin and IGF-1 levels have been shown in the double-blind randomized CHOP trial [12-14].

Milk protein seems to play a key role in growth regulation during early childhood. Protein intake is the main contributor for nutritional regulation of the IGF-I axis [15 16]. Milk protein enhances serum IGF-1 to a greater extent than meat protein [17]. This might explain the more pronounced effect of milk protein compared to other proteins on the later risk of obesity that has been reported [8].

Average protein intake of young children in Europe and other regions is much higher than metabolic requirements. During the second year of life, 30-50% of total daily protein is comprised of dairy products [18 19], indicating particular opportunities to reduce overall protein consumption though modifying dairy protein intake.

Therefore, we designed a randomized controlled trial to examine the role of milk protein intake during the second year of life on child growth and later obesity risk. If a reduction of milk protein during the second year of life has an appreciable effect on growth and obesity development, respective dietary modification may be translated into the practice of toddler feeding.

Main Objective

We aim at evaluating the effect of two iso-energetic milk products for young children with differing protein content on growth during the second year of life.

Secondary Study Objectives

Besides treating the study as an intervention study as described in detail below, the study incorporates a longer follow-up and is also considered a cohort study. Data obtained and produced should be scientifically exploited for explorative analysis specifically addressing the interplay and factors that influence child feeding, growth and development, physical activity, metabolism, and disease prevention.

Methods and analysis

Study design and population

The Toddler Milk Intervention trial (ToMI trial) is designed as a two-arm, parallel, randomized, double blind controlled trial to evaluate toddler milk products with different protein content. The study is conducted at university hospitals in Munich, Germany, and in Tarragona and Reus, Spain.

The target population are healthy children at the age of one year. The children are enrolled if they meet the inclusion and exclusion criteria outlined in Table1.

Intervention

Formula composition

Two investigational formulas are used. Both formulas are based on cow's milk. The protein is unmodified from cow's milk and has the same casein:whey protein ratio in both formulas. The experimental formula contains 0.72g protein/100ml (1.5g/100 kcal), with a protein content that is similar to breast milk in advanced lactation. The control formula contains 2.95g protein/100ml (6.15g/100 kcal) which is comparable to standard 2% cows´ milk. Contents of energy, carbohydrates, vitamins and minerals are very similar for both formulas (Table 2). In order to reach the same energy content in both formulas, the fat content varies between experimental (4.25g fat/100 kcal) and control formula (2.16g fat/100 kcal) but the lipid composition and the ratio of milk fat/vegetable oils is the same. Both formulas were developed and produced by the sponsor for this trial and were not tested in any other studies before the trial.

Dose, route of administration and schedule of formula

Participating families receive the formula as milk powder (one can comprises about 400g of product) and are advised to prepare the formula according to the instructions which were identical for all product codes. It is recommended to consume at least 300ml of formula per

day. Further, parents are encouraged to substitute with the study formula any milk intake from the child´s diet. The intake of other dairy products such as cheese or yoghurt is accepted.

The intervention starts with the first study visit at around one year of age and ends with the third study visit at around two years of age. The study formula is given to the parents at no costs and is delivered directly to subject's home. Subject's compliance is regularly checked by telephone and personal interviews. After the end of the intervention, return and pick-up of remaining cans is organized. If not possible, families are advised to destroy remaining infant formula cans.

Discontinuation criteria

Discontinuation of the trial can be either due to withdrawal of consent at any time or due to the investigator´s decision that continuation within the trial might impair child´s health.

Outcome measurements

Primary endpoint

The primary endpoint is BMI-for-age z-score (based on the WHO Multicentre Growth Reference Study [20]) at the age of 24 months adjusted for BMI-for-age z-score at 12 months of age.

Secondary objectives and endpoints

The secondary objectives serve to evaluate the safety and efficacy of the two milk products used and to complement the primary endpoint. Secondary endpoints will also be adjusted for baseline measurements if available. Secondary endpoints are:

- BMI-for-age z-score at 72 months,
- The percentage of overweight and obese children at 24 months of age according to CDC definition: Overweight is at and above the 85th to less than 95th percentile and obese 95th percentile or greater
- The percentage of overweight and obese children at 72 months of age,
- Anthropometric measures (z-scores for weight, length and head, waist and arm circumference at 12, 18, 24, 48 and 72 months of age; hip circumference at 48 and 72 month of age),
- Subcutaneous fat distribution (from skinfold thickness at 24, 48 and 72 months of age),
- Total body fat and lean mass (from BodPod measurements at 24, 48 and 72 months of age),
- Blood pressure (48 and 72 month of age),
- Child development (24 and 48 months of age),

- Metabolic and endocrine markers (IGF-1, IGF-BP2, IGF-BP3, insulin, leptin, adiponectin, ghrelin, lipid profile and complete blood count at 12, 24 and 72 month of age),
- Serum albumin, urea, creatinine, amino acids at 12, 24, 72 months of age and ferritin and 25-OH-vitamin D (at 24 months of age),
- Metabolic profile (from plasma at 12, 24 and 72 months of age and from urine samples at 12, 18, 24, 48 and 72 months of age),
- Urine markers (Calcium, C-peptide, creatinine urea nitrogen at 12, 18, 24, 48 and 72 months of age),

Furthermore, the following hypotheses will be examined:

- Total energy intake is not affected by the low protein formula.
- Total protein intake is lower in the group of protein reduced formula.
- Plasma concentrations of essential amino acids and of IGF-1 at the age of 24 months are lower in the low protein formula group compared to the high protein formula group.
- Systolic and diastolic blood pressure measurements at the ages of 48 and 72 months are lower in the low protein formula group compared to the high protein formula group.
- Body fat mass at age 24 months is lower in the low protein formula group compared to the high protein formula group.
- DNA methylation affects the association of protein intake and BMI
- Protein intake affects DNA methylation
- DNA methylation affects the association of protein intake and the metabolic profile

DNA methylation is currently only planned as an option provided additional funding can be secured.

Sample size

The sample size calculation is based on the observations from the CHOP-study [1]. This trial examined the difference in BMI-for-age z-scores between two groups of children fed a higher or lower protein content formula during the first year of life. At 24 months of age the BMI for age z-score difference between both formula groups was 0.2 standard deviations (SD). The absolute difference in protein content between intervention and control group in the CHOP-trial was lower (Infant formula: 0.8g/100ml; Follow-on formula: 1.6g/100ml) compared to the ToMI-trial (2.2g/100ml). Despite a higher protein difference, we expect a lower effect of the intervention due to the lower contribution of milk to the total protein intake in the second year of life. Thus, we assume a slightly lower mean difference in BMI for age z-score of 0.15 SD at 24 months of life.

The sample size was calculated with an anticipated effect size on BMI for age z-score of 0.15 SD and a standard deviation of 0.9. Assuming a power of 80 % and a significance level of 5%

(two-sided alpha of 0.05), a sample size of 566 subjects per intervention arm is calculated. Therefore, 1,132 subjects in total are needed. To have enough power to detect also a difference of the same magnitude at 72 months (6 years) of age, at an assumed loss to follow-up of 30%, a final sample size of 1,618 subjects was estimated.

Recruitment

The study sites in Munich, Reus and Tarragona followed somewhat different recruitment strategies due to different local conditions. In Germany all inhabitants are registered in central registries. The public registries provided the study team for this defined research on a regular basis addresses of all families with children in the required age group (about 26,000 per year). These families living in Munich and about 70 surrounding municipalities were contacted once by postal mail and invited to contact the study team if interested in participation in the trial.

In Spain two recruitment strategies were used for both sites covering about 3000 births per year. First, telephone contacts from families who delivered their child at either of the two hospitals were available. These families were contacted directly. Second, recruitment interviews at primary health care centers were conducted. In these primary health care centers, Spanish children are seen for health care examinations and for vaccinations.

Allocation of study formula and blinding

The study formula cans are labelled with one of eight codes. Four codes each are assigned to the intervention or the control group, respectively. The allocation of the codes is performed online by study staff after check of in- and exclusion criteria within the data capture tool (iMedidata, Medidata Balance, New York, USA) using balanced randomization stratified by country. After enrolment of the subject into the trial, study staff dispense the assigned study formula to the study participant along with instructions for formula preparation.

The study is double blinded with all persons involved in local organization and conduct of the study such as study staff, principal investigator, project manager, biostatistician, data manager, trial monitor and laboratory analysts being unaware of the code allocation. After the code break for the primary outcome analysis, subjects will receive a new identification id in the analysis data to hamper the unblinding for above persons in the further follow-up. An emergency code break by an Investigator may be requested only in case of an unexpected serious adverse event (SAE) suspected to be related to the investigational product.

Data collection, management and analysis

Data collection and management

During the intervention period three visits at the hospital are scheduled at 12, 18 and 24 months of age (Figure 1). At baseline socioeconomic data and data on health, growth and nutrition by 24-hours recalls during the first year of life are assessed. At each visit

anthropometric measurements are performed and urine samples are collected. Blood is taken at 12 and 24 months of age. Additionally, at 24 months of age body composition using an air displacement plethysmography (BodPod COSMED, Rome, Italy) as well as physical activity measurement using an accelerometer device (Actigraph wGT3X-BT, Pensacola, FL, USA) is performed. Further, data of child´s development based on parent answers of the Ages & Stages questionnaire (ASQ-3, Brookes Publishing Co., Inc., USA) are collected.

For follow-up, two additional visits are scheduled at 48 and 72 months of age with anthropometric, body composition and physical activity measurements and collection of urine samples and food frequency questionnaires (Eating Habits Questionnaire -EHQ)[21]. Furthermore, socioeconomic data and data on health are updated and data on nutrition behavior is collected. At 48 months of age, the ASQ-3 is used again. Blood is taken at 72 months of age.

The main primary aim of the nutritional assessment during the intervention phase is to see if the intervention groups differ in nutritional intake. Therefore, a 24h-recall is used. While the second year of life is still considered a nutritional transition period, nutrition patterns are more stable between 48 and 72 months of age and analysis of food patterns are more relevant. Therefore, a FFQ is used for the later time points.

During all study visits and at several additional telephone calls between visits, parents are asked for health problems (including adverse events) and compliance. For compliance the intake of study milk and any discontinuation of study milk intake with reasons are determined. The number of consumed cans will be used to determine the average study milk consumption.

Collected data is organized in different databases. To organize and document all contacts with study participants and to coordinate the shipment of the study product, a web-based participant management tool is used (developed jointly with MedSciNet AB, Stockholm, Sweden). In this database, personal data is saved and stored on a secured data server. This database is separated from the other databases which store all medical, nutritional and laboratory data.

All collected health data are primarily captured on paper except data from questionnaires on physical activity and food frequency questionnaires that are entered by families using LimeSurvey (LimeSurvey GmbH, Hamburg, Germany). All other data are transferred from paper into web-based databases. Nutritional data from 24-hours recalls are entered into Nutritics (NUTRITICS LTD, Dublin, Ireland) with nutritional information from the German nutritional database BLS 3.02 and complemented with the nutritional composition from a variety of commercial infant foods and local foods, obtained directly from the label, producer websites or local food composition databases. All other data are entered into iMedidata (New York, USA).

Laboratory samples are processed according to a laboratory SOP. In general, aliquots have 2D barcodes, are scanned, linked with the subject ID and stored into 96-well racks at -80°C for later analysis. Only blood count, lipid status and HbA1c are measured locally on the day of blood sampling.

To ensure data quality, study staff is trained in regular intervals, and procedures are harmonized among study centers by regular contact and monitoring. Furthermore, anthropometric measurements are performed at least twice and data entry is strictly checked for consistency and plausibility by the monitor. Standard operating procedures for all measurements are in place; anthropometric measurements are based on the WHO Growth Standards study [20].

Statistical methods

A statistical analysis plan is created before final code break for the analysis of primary and secondary outcomes. For the statistical analysis, the full analysis dataset (FAS) and the per-protocol-dataset (PP) will be considered. The FAS comprises all randomized subjects who consumed at least one can of investigational product and was considered reasonable and as close as possible to the intention to treat (ITT) ideal as we dealt with a healthy population that participated not for treatment reasons. The PP compromises all subjects included in the FAS and that were compliant with the aimed product consumption (mean consumption of the recommended daily minimum amount of investigational product of 300ml/d). Compliance will be primarily assessed by the number of tins used by the study subject. A Blind Data Review Meeting with participants of the sponsor and the investigators will define specific rules and definitions for lack of compliance. No imputation of missing values is foreseen.

The primary endpoint will be analyzed in the FAS by linear regression (ANCOVA) and corrected for BMI-for-age z-score at baseline, study center and gender. The results of the final model will be compared to further adjusted models and analysis in the PP group; possible effect modification of the primary outcome will be also considered.

Secondary analyses supporting primary objective:

1. BMI-for-age z-score at 72 months.
2. The percentage of overweight and obese children at 24 months of age according to CDC definition: Overweight at and above the 85th to less than 95th percentile and obese 95th percentile or greater.
3. The percentage of overweight and obese children at 72 months of age.

In order to control the experiment wise false positive rate, the listed hierarchy (primary – secondary endpoints) will be maintained in interpreting these outcomes. The incidence of overweight and obese children at 24 and 72 months of age shall be also estimated according to

International Obesity Task Force IOTF definition [22]. The percentage of overweight and obese children will be analyzed by the method of O. Sauzet, et al. [23].

Secondary endpoints include anthropometric measures, dietary and biochemical data. We will use z-scores of WHO growth standards for anthropometry measures at months 12, 18, 24, and 48. We will use a likelihood-ratio test to examine if there is a longitudinal treatment effect. Additionally, treatment differences at each visit will be analyzed using ANCOVA. The ANCOVA approach was chosen so that treatment differences and p-value do not depend on the stage of analysis. A further supportive analysis with a mixed linear model shall be performed at 6 years of age. Fixed effects shall be the intervention group, age, gender, and age times intervention group. The random effects shall be a random intercept and slope.

Dietary data is collected by 24-hours recalls or food frequency questionnaires, which allow us to test for differences in macronutrient intake using ANCOVA. Hence, we are able to analyze if subjects change their dietary habits over time.

Biochemical data is often log-normal distributed. In order to analyze this kind of data properly, we will log-transform the data to achieve approximately normal distributed residuals.

Monitoring

Data monitoring

To ensure safety of the intervention, an interim analysis is planned when 260 subjects have completed the intervention (at 24 months of age). Non-inferiority for weight-for-age z-score has to be shown. This must be the case in both FAS and PP. A non-inferiority boundary for weight-for-age z-score of minus 0.5 SD was chosen according to Onyango et al. [24]. The same model as for the primary analysis is used. To demonstrate non-inferiority, the lower bound of the two-sided 95% confidence interval of the model based treatment difference must be larger than the non-inferiority margin.

If non-inferiority is shown, the study is continued as planned. Otherwise, a second stage interim analysis is performed including the first 390 subjects who have completed the intervention. Furthermore, the safety evaluation will consider endpoints including adverse events, anthropometry, laboratory data and protein intake. Based on the results of the interim analysis and in accordance with the charter of the Data Monitoring Committee, the DMC will recommend either continuing the study as planned or performing the second stage interim analysis. The DMC is independent and consists of expert clinicians and statisticians with no competing interest. The planned interim safety analysis took place in June 2018 and no safety concerns were detected.

Besides the interim analysis, safety is continuously observed by blinded online monitoring of individual growth curves based on the WHO growth charts. If a considerable number of

subjects drop below the median growth curve, an interim analysis will be initiated and the DMC will review unblinded data.

Harms

Any adverse events (AE) which lead to an untoward medical occurrence except for diagnostic and therapeutic non-invasive and invasive procedures will be recorded during the entire intervention period until 30 days after last study milk intake. After these 30 days, only AE´s which are related to the intervention treatment will be recorded. Each AE will be rated according to its severity and its relationship to the study milk. Additionally, severe adverse events (SAE) which e.g. requires inpatient hospitalization will be reported to the safety manager within 24 hours after notice and will be followed up until the outcome is known. A participant insurance is in place.

Monitoring

A commercial monitoring company reviews the process, AE reporting, data capturing and corresponding source data on a regular basis to ensure protocol compliance, accuracy and completeness.

Protocol versions

Issue date: 15.09.2020; version identifier: 5; number of protocol amendments: 5; initial version: 9 March 2016. First modification: 30 March 2016. Besides adaptation from requests of both ethical committees before the start of the study and several minor changes due to misspecifications in the protocol, several clarifications were needed, e.g. to provide more clarity and criteria for study termination before regular completion of the study, clarification in the statistical interpretation of secondary endpoints, addition of new secondary endpoints physical activity and HbA1c, the adaptation to the new European data protection rules in 2018, and a change in exclusion criteria to allow the inclusion of children that are breastfed once per day. Furthermore, an extensive specification of the safety interim analysis after inclusion of 260 children was added in 2018 and more details for collection of AEs separating the collection into two periods, during and after the intervention, were provided.

Ethics and dissemination

Ethical considerations

This study is conducted in compliance with the International Conference on Harmonization (ICH) guidelines and the Declaration of Helsinki and complies with Good Clinical Practice guidelines. Ethics approval was obtained from the ethical committees of the university hospitals at the Ludwig-Maximilian University in Munich, Germany (Projekt Nr. 555-15) and at the Institut d'Investigació Sanitaria Pere Virgili, Reus, Spain (Ref. CEIm IISPV 013/2016.

All protocol amendments were and will be approved by the ethical committee prior to implementation.

Written informed consent is collected by study staff from all legal guardians prior to study inclusion in adherence with regulatory requirements. Each subject receives oral as well as written informed consent in plain language with adequate time in advance to make an informed decision about study participation. The latest informed consent form for both study sites is enclosed in the online supplementary (Supplementary file). All participants re-consented for any additional measurement added to the protocol.

Patient and Public Involvement

The study protocol was primarily developed at a public university hospital without involvement of the sponsor. There was no further public or patient involvement.

Public dissemination and data availability

Study results will be published in peer-reviewed journals and presented on national and international conferences. Study results will also be communicated to participants. Results will be written-up and published by the investigators without help of professional writers. Authorship will depend on relevant contribution to the study. Investigators have full research freedom and have full access to all data. The full study protocol will be made available upon request. The participant-level dataset is not currently planned to be available because consent was not obtained for the sharing of such data from participant's parents / legal guardians or the Institutional Ethics Committees.

Trial status and time course of the trial

The study started to recruit subjects in September 2016 and finished recruitment of 1,625 children in October 2019. The intervention phase will last until October 2020. The database closure for the analysis of the primary outcome is planned for the first quarter of 2021. The follow-up will be completed around October 2025.

Funding, role of the sponsor and investigators

The sponsor has allocated a fixed budget for each study center to recruit and follow the subjects. The sponsor is producing the study product and distributes the study product to the study subjects. The sponsor is funding the monitoring of the study. The primary protocol was outlined by the investigators and was jointly further developed by investigators and sponsor. Data management will be primarily done by the sponsor, except parts of the compliance checks, checks of biosamples and body composition data, as well as nutritional and physical activity data. The primary analysis will be performed by the sponsor. The investigators have to approve the statistical analysis plan and will have full access to all the data. Any published interpretation of the data has to be in mutual agreement between sponsor and investigator

without hampering the research freedom of the investigators. The urinary metabolic profile will be performed by the sponsor, all other laboratory measurements by the investigators. BK is the coordinating principal investigator with VG being his deputy, JE is principal investigator in Spain.

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Authors´ Statement

VG and VJ wrote the manuscript. VG and BK provided the original outline of the protocol; JE, MZ, MG, and DG participated in the design and set-up of the study. BK, JE, MZ, MG, and DG critically revised the content of the manuscript.

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Conflict of interest

The institutions of VG, VJ, BK, JE, MZ, MG receive funding by the sponsor to conduct the study- and DG is employed by the sponsor of the study.

Tables

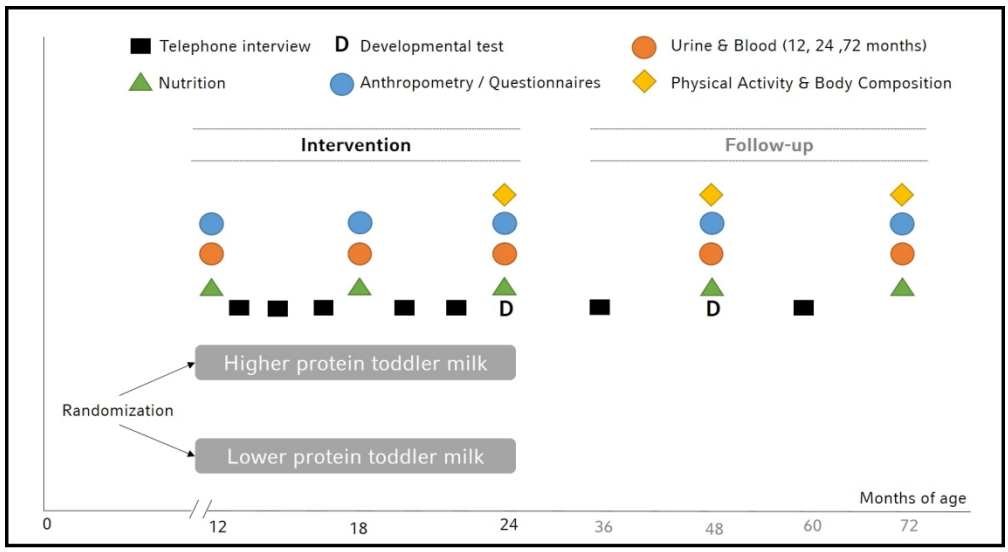
Table 1: Inclusion and Exclusion criteria of the Tomi trial

Inclusion criteria	Exclusion criteria
• Legal guardians signed the written informed consent.	• Infant who is breastfed at least twice in 24 hours at time of enrolment.
• Child was born full term (≥ 37 + 0 weeks of gestation).	• Infant who usually does not drink 300 ml of cow´s milk and/or formula milk per day.
• Child´s birth weight is between 2.5 and 4.5 kg.	• Cow´s milk allergy.
• Child is born from a singleton pregnancy.	• Lactose intolerance.
• Child´s age at enrolment is between 11.5 and 13.5 month.	• Institutionalized children.
• Child´s legal guardians are of legal age and they have sufficient local language skills to understand the study information, informed consent and study procedure.	• Diagnosed disorder, which interfere with nutrition or growth (e.g. celiac disease, inflammatory bowel disease).
• Child and child´s parents are willing to fulfil the requirements of the study protocol and procedures.	• Children who participated in any other interventional clinical trial 4 weeks prior to enrolment.
• Child´s family is available via phone or e-mail throughout the whole study.	

Table 2: Nutritional composition of the interventional products (toddler milks) that are based on cow's milk with the same casein:whey protein ratio.

	Experimental toddler milk (as prepared, per 100ml)	**Control toddler milk** (as prepared, per 100ml)
Energy	201 KJ/48 kcal	201 KJ/48 kcal
Protein	0.72 g	2.95 g
Fat	2.0 g	1.0 g
Saturated fatty acids	0.8 g	0.4 g
Carbohydrates	6.7 g	6.7 g
Lactose	6.7 g	6.6 g
Other	<0.1 g	<0.1 g
Salt	0.1 g	0.1 g
Vitamines		
Vitamine A	71 µg	66 µg
Vitamine D	1.2 µg	1.3 µg
Folic acid	14.9 µg	14.2 µg
Vitamine B12	0.2 µg	0.2 µg
Vitamine C	6.4 mg	6.9 mg
Minerals		
Calcium	115 mg	115 mg
Micronutrients		
Iron	0.5 mg	0.5 mg
Zinc	0.3 mg	0.6 mg

Figure 1: Assessments in children participating in the ToMI trial



Assessments in children participating in the ToMI trial

INFORMACIÓN A LOS PARTICIPANTES

TÍTULO	Efecto de la ingesta de proteínas lácteas en el niño pequeño sobre el crecimiento y el posterior riesgo de obesidad: ensayo clínico aleatorizado
ACRÓNIMO	**TOMI Trial**

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INTRODUCCIÓN: Este documento es informativo sobre el proyecto de investigación que se indica en la cabecera, al cual les invitamos a participar. Les anticipamos que su participación es voluntaria y podrán realizar todas las preguntas que deseen, así como cambiar de opinión sobre su participación en cualquier momento. Su decisión no afectará la calidad de la atención sanitaria que reciba su hijo/a.

OBJETIVO: Este proyecto tiene como objetivo evaluar el efecto de dos fórmulas lácteas de crecimiento (con las mismas calorías, pero con diferente proporción de proteína y grasa) durante el segundo año de vida sobre el crecimiento desde el año hasta los 6 años.

INTERVENCIÓN NUTRICIONAL: Los niños/as de las familias que deseen participar recibirán de forma gratuita una de las dos leches de crecimiento del estudio durante todo el segundo año de vida (50% de probabilidad para cada una). Estas dos leches tendrán el mismo contenido energético (48 Kcal/100 ml) (calorías similares a la leche de vaca semidesnatada) y se diferenciarán en las proporciones de proteínas y grasas. Una de las leches tendrá 2.95g de proteínas y 1.1g de grasas (en 100ml), mientras que la otra tendrá 0.72g de proteínas y 2.11g de grasas (en 100ml). Estas proporciones se encuentran comprendidas entre las proporciones contenidas en la leche materna y la leche de vaca de consumo habitual. En ningún momento del estudio, ni los investigadores ni las familias conocerán cuál de estas leches consume cada participante.

METODOLOGÍA: En este estudio participaran unos 1618 niños de Múnich (Alemania) y Reus/Tarragona. La participación en el estudio tiene una duración de 5 años. Los participantes recibirán una de las dos leches de crecimiento desde el año hasta los 2 años de vida y se evaluará su crecimiento, desarrollo y estado nutricional y de salud a las siguientes edades: 1 año, 1.5 años, 2, 4 y 6 años (en total 5 visitas a lo largo de 5 años). La recogida de datos se llevará a cabo mediante las siguientes evaluaciones y procedimientos en diferentes momentos del seguimiento (que se detallan en la Tabla 1):

- Cuestionarios de salud completados por los padres (o persona a cargo del niño/a)
- Entrevistas telefónicas breves con el equipo de investigación (para revisar la alimentación)
- Exámenes (siempre voluntarios) realizados al niño/a, como:
  - Valoración del crecimiento y la composición corporal a través de medidas antropométricas.
  - Valoración de la composición corporal a través de desplazamiento de aire (se realiza sentado durante pocos minutos en una cámara cerrada llamada “BodPod”).
  - Tensión arterial (a los 4 y 6 años).
  - Actividad física a los 2, 4 y 6 años: la evaluación de la actividad física se realizará mediante cuestionarios específicos, completados por los padres (o persona a cargo del niño/a) y medida a

través de un monitor de actividad física o acelerómetro (Actigraph). El Actigraph es un monitor de actividad física (tipo acelerómetro) que consiste en un pequeño equipamiento médico (peso aproximado: 20gr) que se lleva en la cintura o cadera con un cinturón. Este equipamiento mide la actividad física, el sueño y el gasto energético. El procedimiento consiste en llevar el dispositivo unos 5-7 días para medir la actividad diurna (no hace falta llevarlo por la noche). Después, el dispositivo se retorna al personal del estudio para que extraigan de él los datos.

- Análisis de sangre: la extracción de sangre será realizada por personal cualificado a los 1, 2 y 6 años.
- Análisis de orina: los padres o cuidadores recogerán varias muestras de orina al participante a lo largo del estudio; esta recogida se efectuará mediante una bolsita para lactantes o mediante un tubo convencional de recogida de orina (material que les proporcionará de forma gratuita el equipo investigador) y se entregará en el momento de la visita.

CIRCUNSTANCIAS EN LAS CUALES LA PARTICIPACIÓN DEL SUJETO SE CONSIDERA FINALIZADA: En caso que el participante lo comunique o deje de acudir a las visitas. Mientras el participante no comunique su decisión de dejar de participar, el equipo de investigación seguirá invitándolo a asistir a las visitas. Asimismo, los participantes que no deseen continuar participando en el estudio o que no puedan seguir consumiendo el producto de estudio, serán invitados a acudir a una última visita a los 2 o 6 años.

EFECTOS ADVERSOS: Basados en investigaciones previas, no se espera ningún efecto indeseable por el consumo de la leche de estudio. En cualquier caso, dispondrán de teléfonos de contacto para notificar cualquier incidencia o realizarnos cualquier pregunta. Así mismo, si su hijo/a ha de ser ingresado/a en algún momento por cualquier motivo, rogamos nos lo hagan saber.

RIESGOS: El estudio no supone **ningún riesgo** que no sea el derivado de una extracción sanguínea. Las extracciones de sangre son analíticas normales, que realizará una enfermera con gran experiencia, y pueden causar las molestias propias de un pinchazo. La valoración del volumen corporal a través del desplazamiento de aire es una técnica totalmente segura que no provoca ninguna molestia. El uso del monitor para medir la actividad física no conlleva ningún riesgo. El dispositivo cumple con todos los requisitos de la Unión Europea por lo que respecta a dispositivos médicos de Clase I. En todo momento se tomarán precauciones para evitar al máximo cualquier inconveniente.
De todas formas, pueden seguir participando en el estudio, aunque decidan no realizar alguno de los exámenes anteriormente descritos.

BENEFICIOS: Aunque este proyecto no les promete ninguna ventaja directa, ustedes contribuirán a un mejor conocimiento de la importancia de la alimentación infantil sobre la obesidad infantil y el riesgo de padecer enfermedades cardiovasculares y posiblemente su participación servirá de ayuda a otras personas con estos problemas en el futuro.

DERECHOS DE LOS PARTICIPANTES

USO DE LAS MUESTRAS BIOLÓGICAS: Servirán para llevar a cabo determinaciones bioquímicas, metabólicas, epigenéticas y genéticas relacionadas con el objetivo del estudio (la obesidad y las enfermedades cardiovasculares). En primer lugar, se analizarán parámetros del estado nutricional general, los resultados de los cuales serán comunicados a las familias.
Una parte de las muestras de sangre y las muestras de orina serán enviadas anonimizadas a los laboratorios centrales del proyecto en Múnich (Labor für Stoffwechsel & Ernährung, Hauner Childrens Hospital y Laboratoriumsmedizin, KUM). Otras muestras codificadas pueden ser enviadas a Nestec, en Suiza, o a sus filiales o a terceros para hacer otros análisis. Usted puede decidir restringir el uso de estas muestras para

que no se lleven a cabo análisis genéticos (genes relacionados con la obesidad) indicándolo en la hoja de consentimiento.

Debido a la constante evolución del conocimiento y de las técnicas de investigación en esta área de la salud, es posible que en el futuro pueda realizarse una investigación complementaria relacionada con el objetivo del estudio. Por ello, los posibles sobrantes de las muestras de sangre y orina se preservarán en las mismas condiciones de anonimato y confidencialidad, y en un plazo máximo de 10 años serían destruidas. Ustedes pueden restringir la preservación de estas muestras indicándolo en la hoja de consentimiento. El tratamiento y uso de las muestras se realizará siguiendo lo especificado en la Ley de Investigación Biomédica (14/2007), y en el RD 1716/2011.

PROCEDIMIENTOS NO PLANIFICADOS: Debido a la constante evolución del conocimiento científico y las técnicas, el promotor y sus colaboradores pueden desarrollar análisis no planificados relacionados con los objetivos de este ensayo y/o relacionados con investigaciones futuras en el campo de la salud y/o nutrición. Si ustedes consintieran, las muestras biológicas sobrantes (sangre y orina) o los datos, serán almacenados bajo las mismas condiciones de anonimato y confidencialidad para poder ser reutilizadas en análisis complementarios y/o futuras investigaciones científicas (siempre relacionadas con la asociación entre la alimentación infantil, el crecimiento y la salud). Si ustedes reúsan, las muestras de su hijo/a serán almacenadas por un periodo máximo de 2 años y serán destruidas una vez el estudio y sus análisis estén terminados. Tienen el derecho de limitar el tiempo de retención y uso de estas muestras indicándolo en este consentimiento informado. Si aceptan el uso posterior de los datos y/o las muestras no planificadas en el protocolo inicialmente, serán informados y se les pedirá que den su consentimiento para estos análisis adicionales.

DEFINICIÓN DE DATOS PERSONALES: Datos personales son toda información que se relacione con una persona identificada o identificable. Una persona identificada o identificable es una persona natural que se puede identificar, directa o indirectamente, en particular a través de un identificador como por ejemplo un nombre o un código.

CONFIDENCIALIDAD: Para este estudio, las muestras biológicas obtenidas, así como toda la información recogida se codificarán con un número de forma que no aparezca ni su nombre ni su número de historia clínica. Únicamente los miembros del equipo de investigación tendrán acceso a sus datos y únicamente ellos podrían ponerse en contacto con ustedes y relacionar sus datos personales con los datos de salud recogidos. Para garantizar la calidad y seguridad del estudio, podrán supervisar la recogida de datos de salud: el monitor de calidad, las autoridades sanitarias, un representante autorizado de Nestlé y el Comité Ético de Investigación Clínica.

Se garantiza que todos los datos y resultados obtenidos serán **absolutamente confidenciale**s y que se utilizarán los mecanismos necesarios para el cumplimiento de la "Ley orgánica 15/1999, del 13 de Diciembre" para la protección de datos personales, y la "Ley 14/2007 de Investigación Biomédica ". El equipo de investigación de la *Unitat de Pediatria* de la *Facultat de Medicina de la Universitat Rovira i Virgili* será responsable de sus datos y muestras. El equipo de investigación garantiza su confidencialidad y el hecho que las muestras y los resultados sean utilizados únicamente para las finalidades consentidas. El responsable de sus datos personales codificados (estos datos no contienen ningún nombre o dirección suya o de su familia) es Nestec Ltd., con domicilio en Avenue Nestlé 55, CH-1800, Vevey, en Suiza. Los participantes tienen derecho a acceder, cambiar y oponerse al uso de sus datos, en cualquier momento, simplemente contactando con un investigador (derechos otorgados por Ley 15/1999). Tengan en cuenta que tienen además los derechos de ver y acceder a sus datos, de borrarlos, limitar su procesamiento o la transferencia, presentar una objeción al tratamiento en las circunstancias y los términos especificados en la normativa anterior (derecho concedido por la Ley 15/1999 y 18/2018 Coll., sobre protección de datos de

carácter personal y Reglamento UE 2016/679 del Parlamento Europeo y del Consejo de 27 de abril de 2016). No obstante, el promotor se reserva el derecho de no borrar los datos recogidos antes de retirar su consentimiento y que ya se hayan analizado como parte del estudio. Tienen el derecho de solicitar información sobre los datos del estudio recogidos por los doctores del mismo o por el promotor y sus afiliados (o representantes). Si desean ejercer estos derechos, o presentar una reclamación o solicitar la corrección de cualquier inexactitud de estos datos, pónganse en contacto con el médico del estudio o con el agente de protección de datos del Centro (*Unitat de Recerca en Pediatria i Desenvolupament Humà*. Sant Llorenç 21. 43201 Reus. Telf.977 759364 o 977 759365).

Si decidiesen retirar su consentimiento, solo deberán comunicarlo a los investigadores, en tal caso, no se incorporarán más datos a la base de datos y, si lo desean, también pueden solicitar por escrito la destrucción de sus muestras biológicas. Toda la información recogida en las visitas y exploraciones complementarias se codifica como el resto de muestras y datos del estudio TOMI con un número de forma que aparezca ni su nombre ni su número de historia clínica.

TRANSFERENCIA DE DATOS: Los datos del estudio recogidos de su hijo/a serán enviados al promotor, a terceros que trabajen para el promotor y a las autoridades reguladoras si así lo reclamaran. Solamente datos codificados se almacenarán mediante un sistema informático seguro que pertenece a Medidata, empresa ubicada en todo el mundo, un tercero de Nestlé. El acceso al sistema web está restringido al personal del estudio y a los representantes del promotor. El promotor también podrá utilizar los datos del estudio para poder comercializar la fórmula del ensayo en algunos países o para publicarlos. No obstante, nada que pueda revelar su identidad ni la de su hijo/a saldrá fuera del centro.

Sus datos codificados y los de su hijo/a serán transferidos y procesados a países distintos de España, en condiciones que garanticen su confidencialidad, desde el centro a Nestlé Suiza y otros países/organizaciones internacionales que actúen en nombre del promotor. Como responsable de los datos, Nestlé ha tomado medidas contractuales, organizativas y de seguridad que aseguren el mantenimiento del nivel de protección adecuado exigido por las leyes europeas y españolas, sea cual sea la tercera parte del estudio o los países a los que se transfieran los datos. Durante estos procedimientos no se divulgará su identidad ni la de su hijo/a.

VOLUNTARIEDAD: Su participación en este estudio es totalmente **voluntaria**; pueden decidir no participar, o cambiar su decisión y denegar su consentimiento en cualquier momento, hecho que no afectará ni perjudicará la relación con su médico ni su atención. Para ello, únicamente deberán comunicarlo al equipo de investigación.

INFORMACIÓN SOBRE EL ESTUDIO: Si se dispusiera de nueva información sobre el producto en estudio que pueda influir en su decisión de continuar en el mismo, se les informará de manera oportuna. En el caso de que estas investigaciones proporcionen datos que pudieran ser clínica o genéticamente relevantes para ustedes e interesar a su salud o a la de su familia, les serán comunicados salvo que indiquen expresamente que no desean recibir esta información. Aunque no deseen recibir esta información, tengan en cuenta que la ley establece que, cuando la información obtenida sea necesaria para evitar un grave perjuicio para la salud de sus familiares biológicos, un comité de expertos estudiará el caso y decidirá si es conveniente informar a los afectados o a sus representantes legales. Si por alguna razón ustedes quisieran conocer los resultados de las investigaciones que se hayan producido como consecuencia de su colaboración, podrán ponerse en contacto con los responsables del proyecto, que les informarán debidamente.

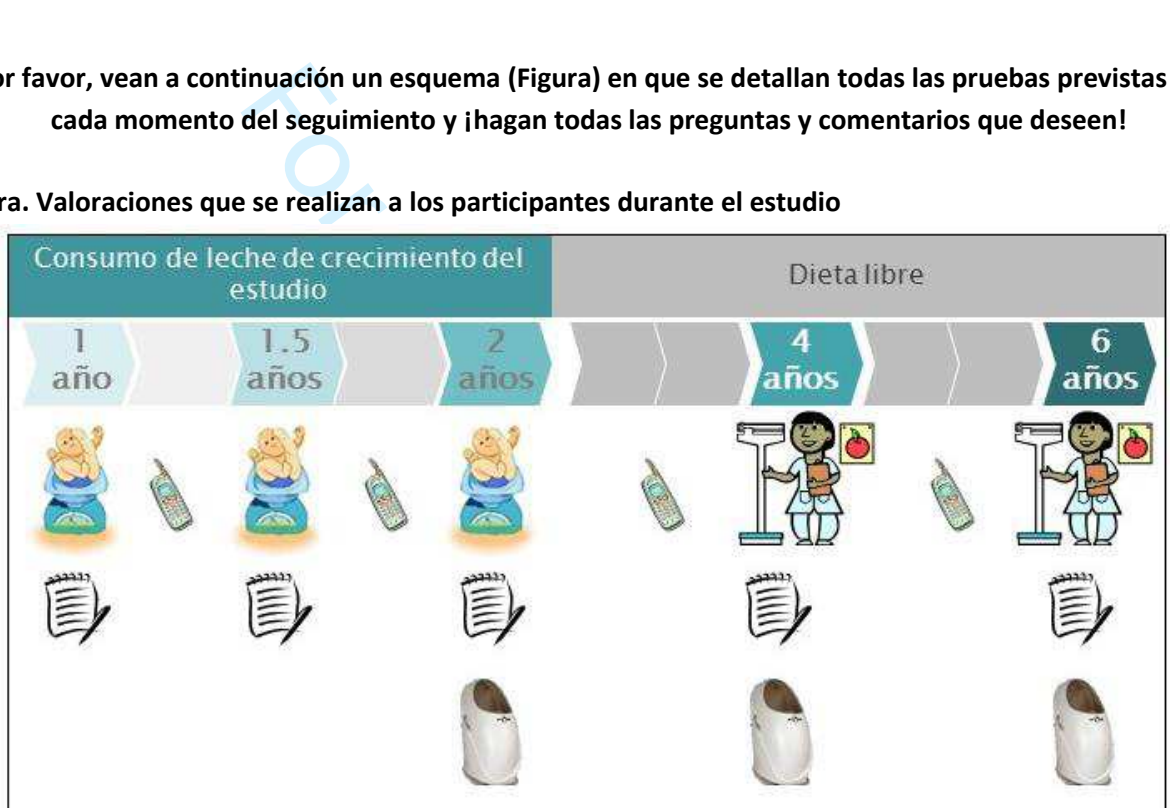
COMPENSACIÓN: Ustedes no recibirán incentivos económicos para participar en el estudio, pero recibirán una compensación que minimice el coste de tiempo y desplazamiento por acudir a la visita.

FONDO DE FINANCIACIÓN: Este estudio recibe soporte económico de Nestec Ltd., Avenue Nestlé 55 CH-1800 Vevey, Switzerland. Esta compañía es tomadora de un **seguro de responsabilidad** (contratado con la compañía Zurich Insurance plc., con nº de póliza Z140955 para el Hospital Universitari de Tarragona Joan XXIII y Z140963 para el Hospital Universitari Sant Joan de Reus) por cualquier posible consecuencia negativa sobre los participantes del estudio por su participación en el estudio. El promotor tiene la potestad de terminar el estudio en cualquier momento.

OTROS ASPECTOS REGULATORIOS: Este estudio ha sido aprobado por los Comités Éticos de Investigación Clínica del Institut d'Investigació Sanitària Pere Virgili y el de la Fundació Jordi Gol i Gorina. El estudio ha sido diseñado de acuerdo a la Declaración de Helsinki, que establece los criterios de investigación biomédica en personas de forma ética.

Por favor, vean a continuación un esquema (Figura) en que se detallan todas las pruebas previstas en cada momento del seguimiento y ¡hagan todas las preguntas y comentarios que deseen!

Figura. Valoraciones que se realizan a los participantes durante el estudio



INFORMACIÓN DE CONTACTO

Unitat de Pediatria, Facultat de Medicina. Universitat Rovira i Virgili. C/ Sant Llorenç 21, 43201 Reus.

Teléfonos: 977759365 / 977759364/ 619733840 (Tarragona)/ 616891314 (Reus)

(Copia para el participante) **CONSENTIMIENTO INFORMADO**

Sr./Sra. ..... informa al padre/madre Sr./Sra. ..... en relación al estudio TOMI.

He sido informado/a sobre los derechos de mi hijo/a y los posibles riesgos y beneficios de su participación en el proyecto:

- La participación de mi hijo/a en este estudio es voluntaria.
- Se garantiza que los derechos y atención sanitaria de mi hijo/a no se verán influenciados por mi decisión.
- Los resultados relevantes que se obtengan a través de este proyecto podrán ser publicados, pero mis datos personales nunca serán revelados a no ser que lo requiera la ley.
- En el caso de que tenga cualquier duda o problema relacionado con el estudio, puedo contactar con Dr. Ricardo Closa, Dr. Joaquín Escribano, Dra. Natalia Ferré y Dra. Verónica Luque a través de los teléfonos 977759364, 977759365, 619733840 (Tarragona), 616891314 (Reus).
- Me ha sido entregada la hoja de información a los participantes y he sido informado/a de la naturaleza del estudio, que se resume en dicha hoja.
- He podido hacer preguntas para aclarar mis dudas.
- Puedo hacer cualquier pregunta en cualquier momento sobre este proyecto.
- He sido informado/a sobre mis derechos como participante en la investigación y, voluntariamente consiento en ceder mi material biológico y genético (muestras de sangre y orina) bajo las condiciones descritas y únicamente para los objetivos definidos.

Respondiendo a las preguntas de abajo declaro que:

- Deseo ser informado de los resultados clínicamente relevantes. Si □ No □
- Accedo a los análisis genéticos y epigenéticos opcionales que se realizaran dentro del marco de este estudio. Si □ No □
- Acepto que las muestras y datos sean utilizados y almacenados más tiempo que para los objetivos concretos del estudio y puedan ser utilizadas en futuros estudios científicos dentro del campo de la salud o la nutrición. Si □ No □
- Si la respuesta a la pregunta 3 es "No": Accedo a que se me contacte en un futuro en el caso de que estime oportuno añadir nuevos datos a los recogidos inicialmente o muestras biológicas adicionales. Si □ No □

Firma del padre/ tutor	**Firma de la madre/ tutor**	**Firma del informador**
Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _
Su firma indica que usted ha leído y entiende la información antedicha, que usted ha discutido este estudio con la persona que obtiene este consentimiento, que usted ha decidido participar basado en la información proporcionada, y que se le ha dado a usted una copia de este formulario. En caso que únicamente uno de los dos progenitores o cuidadores legales esté presente en esta entrevista, su firma implica que el otro progenitor está de acuerdo. De todas formas se le facilitará una hoja de firma para que esta sea entregada en la siguiente visita.		Declaro que los requisitos para el consentimiento informado para este proyecto han sido satisfechos, que he proporcionado al participante una copia de este formulario, discutido con él/ella el proyecto y le he explicado en términos no técnicos la información contenida en este documento. Igualmente, certifico que animé al participante a que hiciera preguntas y que todas fueron contestadas.

(Copia para el investigador) **CONSENTIMIENTO INFORMADO** **ID**:_____

Sr./Sra. ……. informa al padre/madre Sr./Sra. ……..... en relación al estudio TOMI.

He sido informado/a sobre los derechos de mi hijo/a y los posibles riesgos y beneficios de su participación en el proyecto:

- La participación de mi hijo/a en este estudio es voluntaria.
- Se garantiza que los derechos y atención sanitaria de mi hijo/a no se verán influenciados por mi decisión.
- Los resultados relevantes que se obtengan a través de este proyecto podrán ser publicados pero mis datos personales nunca serán revelados a no ser que lo requiera la ley.
- En el caso de que tenga cualquier duda o problema relacionado con el estudio, puedo contactar con Dr. Ricardo Closa, Dr. Joaquín Escribano, Dra. Natalia Ferré y Dra. Verónica Luque a través de los teléfonos 977759364, 977759365, 619733840 (Tarragona), 616891314 (Reus).
- Me ha sido entregada la hoja de información a los participantes y he sido informado/a de la naturaleza del estudio, que se resume en dicha hoja.
- He podido hacer preguntas para aclarar mis dudas.
- Puedo hacer cualquier pregunta en cualquier momento sobre este proyecto.
- He sido informado sobre mis derechos como participante en la investigación y, voluntariamente consiento en ceder mi material biológico y genético (muestras de sangre y orina) bajo las condiciones descritas y únicamente para los objetivos definidos.

Respondiendo a las preguntas de abajo declaro que:

- Deseo ser informado de los resultados clínicamente relevantes. Si □ No □
- Accedo a los análisis genéticos y epigenéticos opcionales que se realizaran dentro del marco de este estudio. Si □ No □
- Acepto que las muestras y datos sean utilizados y almacenados más tiempo que para los objetivos concretos del estudio y puedan ser utilizadas en futuros estudios científicos dentro del campo de la salud o la nutrición. Si □ No □
- Si la respuesta a la pregunta 3 es "No": Accedo a que se me contacte en un futuro en el caso de que estime oportuno añadir nuevos datos a los recogidos inicialmente o muestras biológicas adicionales. Si □ No □

Firma del padre/ tutor	**Firma de la madre/ tutor**	**Firma del informador**
Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _
Su firma indica que usted ha leído y entiende la información antedicha, que usted ha discutido este estudio con la persona que obtiene este consentimiento, que usted ha decidido participar basado en la información proporcionada, y que se le ha dado a usted una copia de este formulario. En caso que únicamente uno de los dos progenitores o cuidadores legales esté presente en esta entrevista, su firma implica que el otro progenitor está de acuerdo. De todas formas se le facilitará una hoja de firma para que esta sea entregada en la siguiente visita.		Declaro que los requisitos para el consentimiento informado para este proyecto han sido satisfechos, que he proporcionado al participante una copia de este formulario, discutido con él/ella el proyecto y le he explicado en términos no técnicos la información contenida en este documento. Igualmente, certifico que animé al participante a que hiciera preguntas y que todas fueron contestadas.

(Copia para el investigador, en caso que se obtenga posteriormente el consentimiento de uno de los dos progenitores)

CONSENTIMENTO INFORMADO **ID**:_____

Sr./Sra. ……. informa al padre/madre Sr./Sra. ……. en relación al estudio TOMI.

He sido informado/a sobre los derechos de mi hijo/a y los posibles riesgos y beneficios de su participación en el proyecto:

- La participación de mi hijo/a en este estudio es voluntaria.
- Se garantiza que los derechos y atención sanitaria de mi hijo/a no se verán influenciados por mi decisión.
- Los resultados relevantes que se obtengan a través de este proyecto podrán ser publicados pero mis datos personales nunca serán revelados a no ser que lo requiera la ley.
- En el caso de que tenga cualquier duda o problema relacionado con el estudio, puedo contactar con Dr. Ricardo Closa, Dr. Joaquín Escribano, Dra. Natalia Ferré y Dra. Verónica Luque a través de los teléfonos 977759364, 977759365, 619733840 (Tarragona), 616891314 (Reus).
- Me ha sido entregada la hoja de información a los participantes y he sido informado/a de la naturaleza del estudio, que se resume en dicha hoja.
- He podido hacer preguntas para aclarar mis dudas.
- Puedo hacer cualquier pregunta en cualquier momento sobre este proyecto.
- He sido informado sobre mis derechos como participante en la investigación y, voluntariamente consiento en ceder mi material biológico y genético (muestras de sangre y orina) bajo las condiciones descritas y únicamente para los objetivos definidos.

Respondiendo a las preguntas de abajo declaro que:

- Deseo ser informado de los resultados clínicamente relevantes. Si □ No □
- Accedo a los análisis genéticos y epigenéticos opcionales que se realizaran dentro del marco de este estudio. Si □ No □
- Acepto que las muestras y datos sean utilizados y almacenados más tiempo que para los objetivos concretos del estudio y puedan ser utilizadas en futuros estudios científicos dentro del campo de la salud o la nutrición. Si □ No □
- Si la respuesta a la pregunta 3 es "No": Accedo a que se me contacte en un futuro en el caso de que estime oportuno añadir nuevos datos a los recogidos inicialmente o muestras biológicas adicionales. Si □ No □

Firma del padre/madre/tutor	**Firma del informador**
Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _
Su firma indica que usted ha leído y entiende la información antedicha, que usted ha discutido este estudio con la persona que obtiene este consentimiento, que usted ha decidido participar basado en la información proporcionada, y que se le ha dado a usted una copia de este formulario.	Declaro que los requisitos para el consentimiento informado para este proyecto han sido satisfechos, que he proporcionado al participante una copia de este formulario, discutido con él/ella el proyecto y le he explicado en términos no técnicos la información contenida en este documento. Igualmente, certifico que animé al participante a que hiciera preguntas y que todas fueron contestadas.

Elterninformation und Einverständniserklärung

Einfluss von Milcheiweiß auf das Wachstum und die spätere Entwicklung von Übergewicht (ToMI-Studie)

Studienregistrierung: NCT 02907502 bei clinicaltrials.gov

Bitte lesen Sie diese Information und Einverständniserklärung sorgfältig durch. Das Studienpersonal wird Ihnen jederzeit alle Fragen beantworten.

Die ToMI-Studie wurde durch die Ethikkommission und den Datenschutzbeauftragten des Klinikum der Universität München geprüft und zustimmend bewertet.

Sie erhalten eine Kopie dieses Schreibens für Ihre Unterlagen.

Leiter der Klinik: Direktor der Kinderklinik und Kinderpoliklinik Prof. Dr. Christoph Klein
öffentl. Verkehr: U3, U6, Bus 58 bis Haltestelle Goetheplatz

Liebe Familie,

wir am Dr. von Haunerschen Kinderspital in München führen eine Studie zum Einfluss von Milcheiweiß auf Gewicht und Wachstum von Kindern durch. Die Studie heißt ToMI-Studie (ToMI von engl. ***to**ddler's **m**ilk **i**ntervention* = Kleinkindermilch Intervention).

Warum führen wir die Studie durch

Die zunehmende Häufigkeit von Übergewicht und Fettleibigkeit (Adipositas) stellt ein großes medizinisches Problem dar. Inzwischen sind alle Altersgruppen davon betroffen, insbesondere auch Klein- und Schulkinder. Wir befassen uns sehr intensiv mit den frühkindlichen Ursachen für diese Entwicklung. Unter anderem leiten wir das weltweit größte Forschungsprojekt zu Auswirkungen der frühkindlichen Ernährung auf die Gesundheit im späteren Leben (http://www.project-earlynutrition.eu). Vor einigen Jahren konnten wir in einer anderen EU-finanzierten Studie („CHOP-Studie") mit Säuglingen zeigen, dass ein niedrigerer Eiweißgehalt in der Säuglingsnahrung während des ersten Lebensjahres dazu beiträgt, dass die Kinder im Schulalter seltener übergewichtig sind.

Bei der ToMI-Studie soll nun untersucht werden, ob sich die gleiche Wirkung durch weniger Milcheiweiß auch im zweiten Lebensjahr zeigt. Dafür wurde speziell eine Kleinkindermilch mit reduziertem Eiweißgehalt hergestellt, die im Vergleich zu herkömmlicher Kleinkindermilch und Kuhmilch deutlich weniger Milcheiweiß enthält.

Neben der Ernährung ist auch das Maß an körperlicher Aktivität in der Kindheit ausschlaggebend für die gesunde Entwicklung eines Kindes. Wir wollen dabei vor allem den Zusammenhang zwischen der frühen Ernährung und dem kindlichen Aktivitätsverhalten untersuchen, aber auch mehr über mögliche Einflussgrößen für das Aktivitätsniveau Ihres Kindes herausfinden.

Studienzweck

Ziel der ToMI-Studie ist es, das Wachstum, die Entwicklung und den Stoffwechsel von Kleinkindern zu untersuchen, die im zweiten Lebensjahr eine eiweißreduzierte Kleinkindermilch erhalten.

Ablauf der Studie (siehe auch Bild 1)

Falls Sie der Teilnahme zustimmen, wird Ihr Kind zufällig entweder der herkömmlichen oder einer eiweißreduzierten Kleinkindermilch zugeteilt. Um die Studienergebnisse nicht beeinflussen zu können, werden weder Sie noch wir erfahren, welche Kindermilch Ihr Kind bekommt. Die Studienmilch soll im 2. Lebensjahr alle anderen Milchgetränke und -nahrungen, somit auch Kuhmilch, ersetzen. Sie erhalten die Studienmilch von uns kostenfrei für das gesamte zweite Lebensjahr. Mit dem zweiten Geburtstag Ihres Kindes endet die Phase, in der Ihr Kind die Studiennahrung bekommt. Insgesamt werden 1618 Kleinkinder an der ToMI-Studie teilnehmen (davon 809 in München und 809 in Reus und Tarragona in Spanien) und vom 1. bis zum 6. (72. Monat) Geburtstag beobachtet.

Im Alter von 12, 18, 24, 48 und 72 Monaten werden wir Ihr Kind im Dr. von Haunerschen Kinderspital sehen. Bei jedem Besuch werden wir Ihr Kind untersuchen und Größe, Gewicht und weitere Körpermaße aufnehmen. Wir werden Ihnen jeweils

Fragen zur Gesundheit und Verhalten Ihres Kindes stellen. Um zu erfahren, wie und wo Ihr Kind aufwächst, werden wir Sie anfangs auch zu Ihrer Herkunft, Ausbildung und Familienstruktur sowie zu Ernährungsgewohnheiten im ersten Lebensjahr befragen. Um zu verstehen wie sich Ihr Kind sonst ernährt, werden wir Sie zu jedem Zeitpunkt fragen, was und wieviel Ihr Kind in den vergangenen 24 Stunden gegessen und getrunken hat. Den Urin Ihres Kindes würden wir gerne jedes Mal untersuchen.

Im Alter von 24 und 48 Monat bitten wir Sie einen Fragebogen zur allgemeinen Entwicklung Ihres Kindes auszufüllen. Ab dem 2. Lebensjahr bestimmen wir die Körperzusammensetzung mittels BodPod®. Die BodPod®-Messung ist eine kurze, unkomplizierte Untersuchung mittels Luftverdrängung zur Bestimmung des Körperfettanteils (http://www.bodpod.com/de/produkte/koerperzusammensetzung).

Im Zuge der Studienbesuche mit 2, 4 und 6 Jahren wollen wir die körperliche Aktivität Ihres Kindes messen. Zusätzlich möchten wir mit Hilfe eines Fragebogens Daten über die körperliche Aktivität von Ihnen und Ihrem Kind sammeln. Die Aktivität wird mit einem Akzelerometer (wGTX3-BT, ActiGraph, Pensacola, USA) gemessen. Der Sensor wird mit Hilfe eines Gummibandes an der Hüfte Ihres Kindes befestigt. Aus den gewonnenen Daten können wir Rückschlüsse auf die tägliche Dauer und Intensität des Bewegungsverhaltens Ihres Kindes ziehen.

Eine Blutabnahme (ca. 6 ml) ist am Anfang und mit 2 und 6 Jahren vorgesehen. Wenn es gewünscht wird, können wir zuvor etwas Emla® Crème auf die Haut Ihres Kindes auftragen, um die Einstichstelle örtlich zu betäuben.

Wir werden Sie zusätzlich alle 2-6 Monate kontaktieren, Sie anfangs zum Verzehr der Studiennahrung befragen und uns kurz nach dem Wohlbefinden Ihres Kindes erkundigen.

Weitere Informationen zur Studie finden Sie auch auf unserer Homepage unter http://www.klinikum.uni-muenchen.de/de/forschung/TOMI-Studie.html.

Eine Beschreibung der Studie steht auch unter http://www.clinicaltrials.gov zur Verfügung.

Die Studiennahrung wurde von der Firma Nestec (Avenue Nestlé 55, CH - 1800 Vevey, Schweiz) für die Studie entwickelt und produziert. Die Nahrung entspricht den europäischen Richtlinien und industriellen Standards. Sie enthält 48 kcal / 100ml Energie und 0,7 g / 100ml bzw. 3,0 g / 100ml Eiweiß in der Eiweiß-reduzierten bzw. der herkömmlichen Kindermilch. Sie ist geeignet für die Ernährung von Kleinkindern im Alter von 12 bis 24 Lebensmonaten und darf nur in diesem Zeitraum durch das Studienkind konsumiert werden.

<u>Familienkost, Beikost und Getränke</u>

Natürlich darf Ihr Kind auch während der Studie seine gewohnte Kleinkinderkost bzw. Familienkost zu sich nehmen. Wir bitten Sie nur, die Milchmahlzeiten Ihres Kindes durch Studiennahrung zu ersetzen. Auch die Herstellung von Breimahlzeiten, Puddings oder ähnlicher milchhaltiger Speisen soll möglichst mit der Studienmilch erfolgen. Nach dem 2. Geburtstag sind Sie völlig frei bei der Ernährung Ihres Kindes.

<u>Nutzen und Risiken bei der Teilnahme an der Studie</u>

Durch die Teilnahme an dieser Studie bekommt Ihr Kind die Möglichkeit, eine neuartige Kleinkindermilch zu verzehren. Die Kleinkindermilch wird nach europäischen Richtlinien und industriellem Standard hergestellt. Die neuartige Kleinkindermilch enthält ausreichend Eiweiß und ist im Eiweißgehalt vergleichbar mit Muttermilch. Trotzdem kann es zu Unverträglichkeiten bei Ihrem Kind kommen. Wir erwarten jedoch keine Reaktionen, die über das normale Maß bei Verwendung von Kleinkindermilch hinausgehen.

Eine Teilnahme an der Aktivitätsmessung kann wichtige Hinweise auf das Aktivitätsverhalten Ihres Kindes liefern. Sie erhalten nach der Abgabe des Akzelerometers eine individuelle Einschätzung, welche Ihnen hilft, das Aktivitätsniveau Ihres Kindes besser zu verstehen und ggf. gezielt zu fördern.

Auch wenn das Gerät sehr robust ist und in der alltäglichen Nutzung nicht beschädigt werden kann, ist jedoch bei grober Gewalt die Ablösung von Kleinteilen möglich, die verschluckt werden können.

Das Risiko bei der Blutentnahme ist verschwindend gering. Es ist möglich, dass es zur Bildung eines blauen Flecks und in den seltensten Fällen zu Infektionen an der Einstichstelle kommt.

Falls im Verlauf der Studie wichtige neue Erkenntnisse bekannt werden, die sich auf Ihre Entscheidung über die weitere Teilnahme an dieser Studie auswirken könnten, werden Sie darüber umgehend informiert. Sie erhalten ggfs. eine neue Elterninformation und Einverständniserklärung zum Unterzeichnen, sofern Sie weiter an der Studie teilnehmen möchten.

Sie können aus der Studie ausgeschlossen werden, wenn es medizinische oder organisatorische Gründe notwendig machen. In diesem Falle werden wir Sie darüber informieren und die bis dahin erhobenen Daten anonymisiert verwenden.

Laboruntersuchungen

Blutwerte liefern wichtige Informationen, um die Auswirkungen der Ernährung auf den Stoffwechsel des Körpers beurteilen zu können. Entscheidend sind für uns aber nicht die einzelnen Werte Ihres Kindes – wie bei Krankheiten oder der Bewertungen durch Ihren Kinderarzt -, sondern der Mittelwert von allen ToMI-Kindern. Das bedeutet: Es sollten möglichst alle Kinder mitmachen, damit wir tatsächlich neue Erkenntnisse aus dem Blut Ihres Kindes gewinnen können! Daher hoffen wir sehr, dass Sie einer Blutentnahme bei Ihrem Kind zustimmen. In den Blut und Urinproben führen wir neben Routineuntersuchungen zur Gesundheit (z.B. Blutbild) vor allem Messungen von Stoffen durch, die mit der Eiweiß- und Energieverwertung (z.B. Harnstoff, Glukose, Blutfette) zusammenhängen. Daneben werden Hormone, die mit Wachstum und Gewichtsentwicklung im Zusammenhang stehen, bestimmt. Wir werden Sie über das Blutbild sowie die Untersuchung von Blutfetten informieren. Alle anderen Blutwerte werden erst am Ende der Studie bestimmt und dienen ausschließlich wissenschaftlichen Zwecken.

Um die Proben zu verschlüsseln, werden sie statt mit dem Namen Ihres Kindes mit einem „Pseudonym" versehen. Das Pseudonym ist eine Kombination aus Buchstaben und Zahlen. Nur mit Hilfe von Computerprogrammen (Pseudonymisierungsschlüssel), die Kind und Pseudonym einander zuordnen, kann herausgefunden werden, welche

Probe zu welchem Kind gehört. Der Pseudonymisierungsschlüssel wird nicht an Dritte weitergegeben.

Da in der Forschung ständig neue Erkenntnisse gewonnen werden, bitten wir Sie um die Erlaubnis, eventuell überschüssige Blutproben anonymisiert (eine Zuordnung zu Ihrem Kind ist nicht mehr möglich) bis zu 10 Jahre nach Studienende aufbewahren zu dürfen, damit Blut nicht vergeudet und noch für künftige, innovative Analysen zur Verfügung steht.

Genetische Untersuchungen

Eine Frage die uns beschäftigt ist, wie Veränderungen am Anfang des Lebens (in dieser Studie eine Veränderung der Ernährung im 2. Lebensjahr) den Stoffwechsel und die Gesundheit später beeinflussen können. Eine Möglichkeit, warum es zu einer langfristigen, eventuell lebenslangen Prägung kommen könnte, sind Veränderungen in der Steuerung der Genaktivierung. Während man vor kurzem noch glaubte, dass man Erbfaktoren, also Gene, einfach hat oder nicht hat, weiß man heute viel mehr, wie Gene „an- und ausgeschaltet" werden können („Epigenetik"). Durch eine Untersuchung der Erbsubstanz im Blut können wir feststellen, welche für den Stoffwechsel, die Körperzusammensetzung, Übergewicht und damit einhergehende Erkrankungen relevante Gene an- oder ausgeschaltet wurden.

Wenn Sie der Untersuchung zustimmen, wird aus einer Blutprobe Ihres Kindes die Erbsubstanz (DNA) gewonnen und untersucht. Die Blutproben werden im Alter von 12, 24 und 72 Monaten gesammelt, um Veränderungen in der Steuerung der Gene feststellen zu können. Die eigentlichen genetischen Untersuchungen erfolgen erst zu einem späteren Zeitpunkt, wenn von möglichst allen Probanden die DNA zu den drei genannten Zeitpunkten gewonnen wurde.

Für die Genuntersuchung muss keine zusätzliche Blutprobe abgenommen werden. Es wird das „Abfallprodukt" der übrigen Blutproben verwendet, die abgetrennten Blutzellen, die ansonsten für keine Untersuchung genutzt werden können. Aus diesen Zellen wird die Erbsubstanz (DNA) gewonnen und die meisten der bisher bekannten, informationsenthaltenden Abschnitte des Erbguts untersucht. Anhand dieser Informationen können wir feststellen, welche Gene an- und ausgeschaltet wurden, die für Stoffwechsel, Körperzusammensetzung und Übergewicht sowie die assoziierte Erkrankungen relevant sind. Außerdem können wir diese Veränderungen in Zusammenhang mit den vielen Einflüssen betrachten, die wir im Rahmen der Studie bei Ihrem Kind beobachten.

Aus der Untersuchung von Erbfaktoren und deren Aktivität ergibt sich für Ihr Kind kein direkter Vorteil. Mit Ihrer Teilnahme unterstützen Sie jedoch die Forschung, wie frühkindliche Ernährung und Verhaltensweisen sowie Umweltfaktoren andauernde Veränderungen verursachen. Dadurch kann möglicherweise die Grundlage für Verbesserungen in der Diagnose und Behandlung von Erkrankungen gelegt werden.

Die Untersuchungen auf Erbfaktoren werden pseudonymisiert bzw. in irreversibel anonymisierter Form am Helmholtz-Zentrum München, Institut für Molekulare Epidemiologie durchgeführt. Durch eine doppelte Kodierung (den pseudonymisierten Proben wird vor der Aufarbeitung eine fortlaufende Labor-Nummer zugeordnet) ist es den Mitarbeitern des Helmholtz-Zentrums nicht möglich, Rückschlüsse auf die persönlichen Daten des Probanden zu ziehen. Damit ist sichergestellt, dass diese

besonders sensiblen genetischen Daten zusätzlich geschützt werden. Die genetischen Untersuchungen werden nur für Forschungszwecke im Rahmen der ToMI-Studie durchgeführt. Es ist nicht möglich und nicht vorgesehen Ergebnisse mitzuteilen. Die statistische Auswertung der genetischen Daten wird unter Verantwortung von Prof. B. Koletzko durchgeführt, ohne Bezug zum Namen Ihres Kindes.

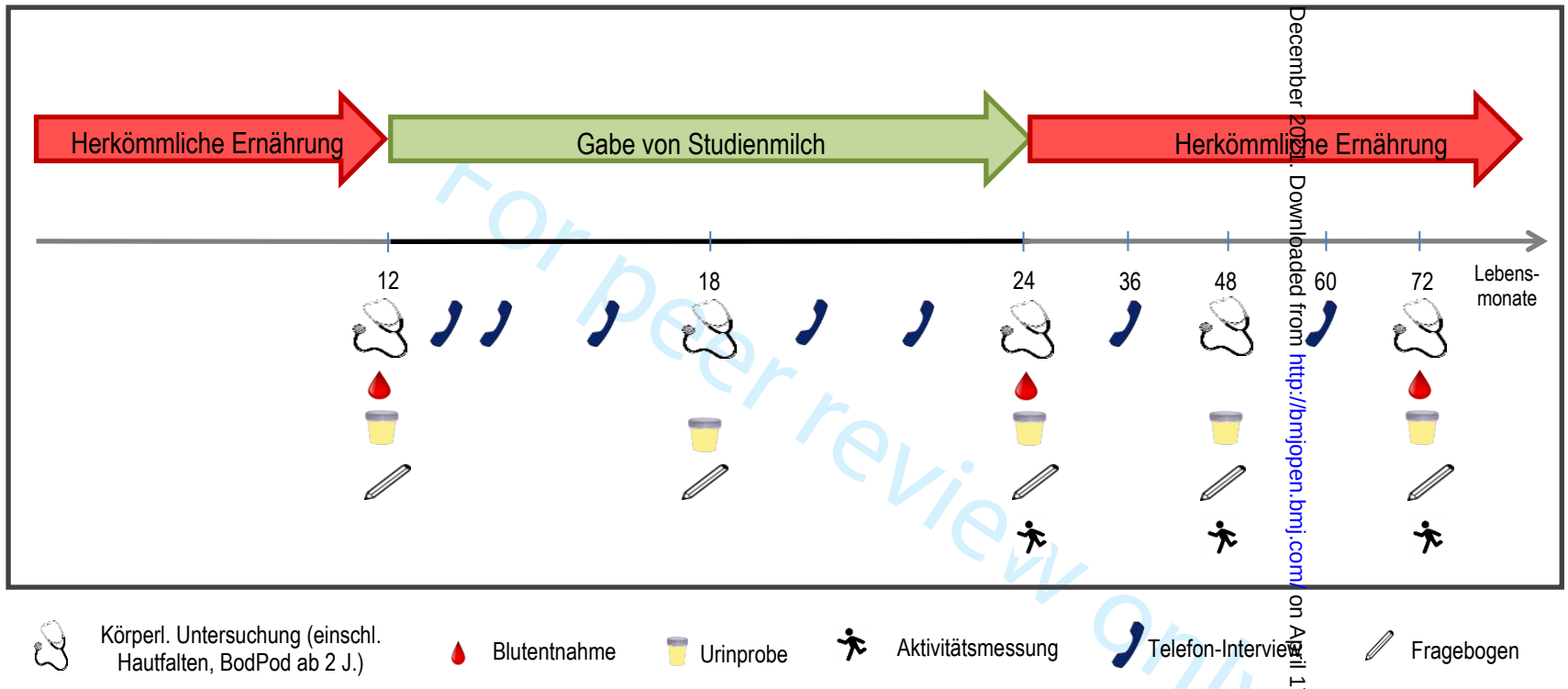


Bild 1. Ablauf der Studie

Studienauswertung

Die Daten, Proben und Fragebögen werden ausschließlich für den oben genannten Studienzweck verwendet. Die Studienauswertung wird gemeinsam mit Nestec durchgeführt. Die Veröffentlichung von Ergebnissen und deren Interpretation erfolgt einvernehmlich.

Studienfinanzierung

Die Studie wird durch die Firma Nestec Ltd. (Avenue Nestlé 55, CH - 1800 Vevey) finanziert. Die Finanzierung umfasst das nötige Studienpersonal, Laboruntersuchungen und die Studiennahrung. Weitere wissenschaftliche Untersuchungen werden durch öffentliche und gegebenenfalls private Finanzierungen erfolgen.

Versicherungsschutz

Auch wenn keinerlei Komplikationen erwartet werden, so sind doch alle Studienteilnehmer durch eine Studienversicherung abgesichert. Der Versicherungsschutz erstreckt sich auf alle Gesundheitsschädigungen, die als Folge der im Zusammenhang mit der Studie angewendeten Maßnahmen eintreten bis zu einer Höchstsumme von € 5.000.000.
Im Schadensfalle können Sie sich direkt an den Versicherer (Zurich Insurance plc NfD, Solmsstraße 27-37, 60486 Frankfurt am Main, Tel.: 069 7115-0; Policen-Nummer: 801.380.024.996) wenden und Ihre Ansprüche geltend machen. Um den Versicherungsschutz nicht zu gefährden, müssen Sie folgendes beachten:

- Teilen Sie uns alle medizinischen Behandlungen mit, denen sich Ihr Kind während der Studienphase unterzieht (Ausnahmen sind Vorsorgeuntersuchungen und Impfungen). Dies gilt auch für die Einnahme neuer Medikamente.
- Teilen Sie eine Gesundheitsschädigung, die als Folge der Studienteilnahme eingetreten sein könnte, bitte dem zuständigen Studienpersonal und der oben genannten Versicherungsgesellschaft mit.

Freiwilligkeit / Rücktrittsklausel

Die Teilnahme an der Studie ist freiwillig. Mit Ihrer Einwilligung auf der „Einverständniserklärung" geben Sie Ihr Einverständnis zur Teilnahme Ihres Kindes an dieser Studie. **Sie haben das Recht, zu jeder Zeit ohne Angabe von Gründen und ohne Nachteile die Teilnahme an der Studie zu beenden**.

Aufwandentschädigung

Für die Teilnahme an der Studie erhalten Sie eine Aufwandsentschädigung.

Wenn Sie weitere Fragen zu dieser Studie haben oder wenn Sie der Ansicht sind, eine studienbezogene Gesundheitsschädigung erlitten zu haben, stehen wir Ihnen gern zur Verfügung: Dr. V. Grote, V.Jäger, M. Meier, S. Vogt, N. Antl, und P. Becker.
Tel:089-4400-57427; E-Mail: Tomi.Studie@med.uni-muenchen.de

Datenschutz: Im Rahmen der Studie gelten folgende Regeln des Datenschutzes.

Datenschutz

Bei dieser Studie werden die Vorschriften über die ärztliche Schweigepflicht und den Datenschutz entsprechend den europäischen, deutschen und bayerischen Richtlinien und der Deklaration von Helsinki eingehalten. Um Sie kontaktieren zu können, werden Ihre Kontaktdaten in einer Datenbank (MedSciNet, Stockholm, Schweden, http://medscinet.com/) gespeichert. In dieser Datenbank werden persönliche, jedoch keinerlei medizinischen Daten gespeichert. Zur Auslieferung der Studiennahrung erfolgt eine Weitergabe Ihrer Adressdaten an ein externes Logistik-Unternehmen (OCasa Lodilat Logistica S.L., Avda de la Astronomia 8, 28830 San Fernado de Henares, Spain). Eine Weiterverwendung dieser Daten zu anderen Zwecken als der Auslieferung der Studiennahrung ist dem Unternehmen untersagt. Das Unternehmen unterliegt den deutschen gesetzlichen Datenschutzbestimmungen.

Alle weiteren Daten – also „medizinische Daten" -, die nicht der Kontaktaufnahme und Kontaktorganisation dienen, werden in getrennten Datenbanken (Medidata Solutions, 350 Hudson St, New York, NY 10014 sowie lokal im Klinikum der Universität München)gespeichert. Persönliche Daten wie Name oder Adresse werden in diesen Datenbanken nicht erfasst. Die Zuordnung zum Namen Ihres Kindes kann nur über einen Verschlüsselungscode erfolgen, der nur unter aktiver Hilfe des Studienpersonals einem Namen zugeordnet werden kann. So sind alle erhobenen Daten und Befunde Ihres Kindes pseudonymisiert.

Sie haben das Recht, jederzeit Auskunft über Ihre gespeicherten personenbezogenen Daten zu erhalten, diese zu berichtigen oder ggf. löschen zu lassen. Verantwortlich für die Datenverarbeitung ist Prof Dr. Berthold Koletzko sowie Dr. Veit Grote als dessen Stellvertreter.

Kontaktdaten der Datenschutzbeauftragten:

Bei Beschwerden haben Sie das Recht sich an die jeweilige Datenschutz-Aufsichtsbehörde zu wenden.

Der lokale Datenschutzbeauftragte für das Klinikum der Universität München ist:

Herr Gerhard Meyer
Klinikum der Universität München
Pettenkoferstr. 8
80336 München
E-Mail: datenschutz@med.uni-muenchen.de

Die übergeordnete Behörde für die LMU und das Klinikum ist:

Bayerischer Landesbeauftragter für den Datenschutz (BayLfD)
Postanschrift: Postfach 22 12 19, 80502 München
Hausanschrift: Wagmüllerstr. 18, 80538 München
Tel.: 089 212672-0
Fax: 089 212672-50

Datenzugang:

Der Zugang zu den Adressdaten und zum Verschlüsselungscode ist auf folgende Personen der Studienorganisation beschränkt: Prof. B. Koletzko, Dr. V. Grote, V. Jäger, M. Meier, S. Vogt, N. Antl, P. Becker und U. Handel. Weitere Personen aus dem Studienzentrum (Dr. von Haunersches Kinderspital, Abt. Stoffwechsel und Ernährungsmedizin unter der Leitung von Prof. B. Koletzko) können zur Studienorganisation im Verlauf der Studie nach Zustimmung der Studienleitung Zugang erhalten. Die Firma Nestec hat darüber hinaus die Firma PAREXEL International GmbH beauftragt, die Qualität der Studie vor Ort zu überwachen (sog. „Monitoring"). Das Unternehmen wird zum Datenschutz verpflichtet und hat vor Ort Zugang zu persönlichen und medizinischen Daten. Eine Entschlüsselung einzelner Studienteilnehmer erfolgt lediglich in Fällen, in denen es die Sicherheit erfordert („medizinische Gründe"). Das Unternehmen unterliegt den deutschen, gesetzlichen Datenschutzbestimmungen.

Die Firma Nestec hat kontinuierlichen Zugang zu pseudonymisierten Daten, jedoch nie zu den Kontaktdaten. Diese pseudonymisierten Daten werden von Nestec auch in anderen Ländern als Deutschland oder der Schweiz (Sitz von Nestec) verarbeitet. Hierbei wird Ihre Identität gewahrt und die Vertraulichkeit Ihrer Daten gewährleistet. Es gelten für diese Drittländer /internationale Organisationen vertraglich die europäischen und deutschen gesetzlichen Datenschutzbestimmungen.

Einige Stoffwechseluntersuchungen werden in den Laboratorien der Firma Nestec, Avenue Nestlé 55, CH - 1800 Vevey, Schweiz durchgeführt. Die genetischen und epigenetischen Analysen werden in Zusammenarbeit mit dem Helmholtz-Zentrum, Institut für Molekulare Epidemiologie, München erstellt. Alle anderen Untersuchungen werden in Laboratorien des Klinikums der Universität München durchgeführt. Die Blutproben werden hierzu nur mit dem Verschlüsselungscode weitergegeben und lassen keinen direkten Rückschluss auf den Studienteilnehmer zu. Für die genetischen und epigenetischen Analysen wird eine erneute 2. Verschlüsselung durch die Mitarbeiter des Helmholtz-Zentrums durchgeführt. Diese doppelte Kodierung stellt sicher, dass die genetischen und epigenetischen Daten zusätzlich geschützt werden. Eine Entblindung ist nur durch das Studienzentrum, nicht aber durch die Mitarbeiter des Helmholtz-Zentrums möglich.

Im Falle des Widerrufs der Einwilligung werden der Name und Ihre persönlichen Kontaktdaten aus unserer Datenbank gelöscht. Die bis dahin gespeicherten Daten Ihres Kindes werden nun anonymisiert verwendet. Außerdem werden die Kontaktdaten aller Studienteilnehmer innerhalb eines Monats nach Abschluss der Studie gelöscht. Die schriftlichen Unterlagen, inklusive dieser Einverständniserklärung, werden im Dr. von Haunerschen Kinderspital bis zum Ende der Studie und in einem dafür geeigneten Lager bis zum Ablauf der gesetzlichen Aufbewahrungsfrist (12 Jahre nach Studienende) aufbewahrt.

Im Falle von Veröffentlichungen der Studienergebnisse bleibt die Vertraulichkeit der persönlichen Daten Ihres Kindes ebenfalls gewährleistet, denn die Daten werden, wenn überhaupt, in anonymisierter Form wiedergegeben.

Auf Wunsch werden wir Sie über allgemeine Studienergebnisse informieren.

Im Falle von zusätzlichen, bisher nicht geplanten Untersuchungen oder Datenerhebungen, die über den oben genannten Studienablauf hinausgehen, werden wir das zustimmende Votum der zuständigen Ethikkommission einholen.

Leiter der Klinik: Direktor der Kinderklinik und Kinderpoliklinik Prof. Dr. Christoph Klein
öffentl. Verkehr: U3, U6, Bus 58 bis Haltestelle Goetheplatz

Vor der Einwilligung in die Studie haben Sie hier die Möglichkeit gezielt Fragen zu notieren, die noch ausführlicher mit Ihnen besprochen werden sollen.

Einverständniserklärung & Datenschutzerklärung für die Teilnahme meines/unseres Kindes

Einfluss von Milcheiweiß auf das Wachstum und die spätere Entwicklung von Übergewicht (ToMI-Studie)

Name, Vorname des Kindes	Geburtsdatum

Ich erkläre, dass mir die Studienbedingungen vollständig erläutert wurden und alle Fragen zu meiner Zufriedenheit geklärt wurden. Das Formblatt mit den Studieninformationen habe ich erhalten. Ich hatte ausreichend Zeit, dieses Formblatt zu lesen und Fragen zu stellen. Mögliche Risiken und Nachteile für mein Kind wurden mir erklärt. Ich weiß, dass ich jetzt und in Zukunft jede Frage bezüglich dieser Studie und der Untersuchungen stellen kann.
Ich weiß, dass ich/mein Kind jederzeit von der Teilnahme an der Studie zurücktreten kann, ohne dass ich dafür Gründe angeben muss oder dass mir oder meinem Kind Nachteile entstehen würden.

Hiermit willige ich in die Teilnahme meines Kindes in die Studie ein:

Ort, Datum	Name, Vorname 1. Erziehungsberechtigte/r	Unterschrift 1. Erziehungsberechtigte/r

Ich besitze das alleinige Sorgerecht: ☐ Ja ☐ Nein

Ort, Datum	Name, Vorname 2. Erziehungsberechtigte/r	Unterschrift 2. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname Studienpersonal (Aufklärende/r)	Unterschrift Studienpersonal (Aufklärende/r)

Die Datenschutz-Information im Rahmen der Teilnehmerinformation habe ich zur Kenntnis genommen. Ich willige hiermit in die Erhebung und Verwendung der persönlichen Daten meines Kindes nach diesen Maßgaben ein.

Ort, Datum	Name, Vorname 1. Erziehungsberechtigte/r	Unterschrift 1. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname 2. Erziehungsberechtigte/r	Unterschrift 2. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname Studienpersonal (Aufklärende/r)	Unterschrift Studienpersonal (Aufklärende/r)

Einverständnis- & Datenschutzerklärung für die genomweite Genotypisierung und epigenetische Untersuchungen meines/unseres Kindes im Rahmen der ToMI-Studie

Einfluss von Milcheiweiß auf das Wachstum und die spätere Entwicklung von Übergewicht (ToMI-Studie)

Name, Vorname des Kindes	Geburtsdatum

Hiermit willige ich insbesondere ein, dass aus dem Blut meines Kindes **Erbmaterial gewonnen, aufbewahrt und untersucht** werden darf. Die genomweite Genotypisierung, sowie die epigenetischen Untersuchungen dienen der Aufdeckung genetischer Ursachen von Erkrankungen und Ursachen für Übergewicht und Stoffwechselveränderungen im Rahmen der ToMI-Studie. Die Teilnahme an der Untersuchung birgt keine weiteren gesundheitlichen Risiken über die erfolgende Blutentnahme hinaus.

Die Daten und Untersuchungsergebnisse werden ausschließlich für das Untersuchungsziel dieser Studie verwendet. Auf die verschlüsselten Daten können nur autorisierte Mitarbeiter der Studie zugreifen. Eine Weitergabe von Daten an unberechtigte Dritte erfolgt nicht. Die im Rahmen dieser Studie gewonnenen genetischen Daten werden bis zu 10 Jahren nach Abschluss der wissenschaftlichen Untersuchung oder bis auf Widerruf aufbewahrt.

Ich weiß, dass ich jetzt und in Zukunft weitere Fragen bezüglich dieser Studie und den einzelnen Untersuchungen stellen kann. Ich weiß, dass ich jederzeit von der freiwilligen Teilnahme an der Studie zurücktreten kann, ohne dass ich hierfür Gründe angeben muss.

Ich willige freiwillig in die Erhebung, Verarbeitung und Nutzung personenbezogener Daten nach Maßgabe des Aufklärungsbogens der Studie ein. Für die Erhebung, Verarbeitung und Nutzung ist der Leiter des Forschungsvorhabens, Herr Prof. Berthold Koletzko, verantwortlich.

Ort, Datum	Name, Vorname 1. Erziehungsberechtigte/r	Unterschrift 1. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname 2. Erziehungsberechtigte/r	Unterschrift 2. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname Studienpersonal (Aufklärende/r)	Unterschrift Studienpersonal (Aufklärende/r)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Check/page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Y
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	10
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 13
	5b	Name and contact information for the trial sponsor	13
	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	13
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13
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
	6b	Explanation for choice of comparators	
Objectives	7	Specific objectives or hypotheses	4
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, noninferiority, exploratory)	4

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
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)	4, Table
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4, Table
	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)	5
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
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 relevance of chosen efficacy and harm outcomes is strongly recommended	5,6,
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)	7
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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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	7

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	
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
Methods: Data collection, management, and analysis			
Data collection methods	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 validity, if known. Reference to where data collection forms can be found, if not in the protocol	7,8
	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
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	8,10
Statistical methods	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	8,9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
	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)	8
Methods: Monitoring			
Data monitoring	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	10

	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	10
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	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	10
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	11
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)	11
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	yes
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	8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
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	10
Dissemination policy	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	11
	31b	Authorship eligibility guidelines and any intended use of professional writers	11
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplement
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	No

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effect of milk protein content in toddler formula on later BMI and obesity risk: Protocol of the multicentre randomized controlled Toddler Milk Intervention (ToMI) trial

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Effect of milk protein content in toddler formula on later BMI and obesity risk: Protocol of the multicentre randomized controlled Toddler Milk Intervention (ToMI) trial

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Abstract

Introduction Reduction of milk protein content in infant formula provided during the first year of life has been shown to reduce early weight gain and obesity later in life. While rapid weight gain during the first two years of life is one of the strongest early predictors of obesity, the role of animal protein intake beyond the first year of life is unclear. The aim of this study is to examine the role of milk protein during the second year of life in healthy children on weight gain and obesity risk in preschool age.

Methods and analysis This randomized, double-blinded study enrolled 1,618 children aged 11.5 to 13.5 months in Spain and Germany into 2 groups receiving isocaloric toddler milk with differing protein content during the second year of life. The experimental formula contains 1.5g/100kcal and the control formula 6.15g/100kcal protein and otherwise equal formula composition, except for modified fat content to achieve equal energy density. The primary endpoint is BMI-for-age z-score at the age of 24 months adjusted for BMI at 12 months of age. The children are followed until 6 years of age.

Ethics and dissemination Ethics approval was obtained from the ethical committees of the LMU University Hospital Munich, Germany (Nr. 555-15) and at Institut d'Investigació Sanitaria Pere Virgili, Reus, Spain (Ref. CEIm IISPV 013/2016). We aim at publishing results in peer-reviewed journals and sharing of results with study participants.

Trial registration number NCT02907502

Strengths and limitations of this study

- This study uses a randomized and double blinded design to minimize potential confounding and biases.
- The multicentre design of this study with sites in Spain and Germany increases external validity of study results.
- The follow-up of the cohort is planned until six years of age and will provide the possibility to examine long-term effects of the intervention.
- Conclusions will be limited to effects of dairy protein provided with milk based drinks in the second year of life and cannot be extrapolated to effects of total dietary protein supply.

Keywords

Toddler milk; milk protein; protein intake; clinical trial; obesity; BMI

Introduction

A randomized double blind controlled clinical trial demonstrated that reducing protein intake in infant formula provided in the first year of life lowers early weight gain until 2 years of age [1]. Data from the same study (CHildhood Obesity Project [CHOP] trial) demonstrated that lower protein supply with formula fed in the first year of life also reduced BMI and obesity risk at school age [2]. The results of the CHOP trial contributed to enhanced promotion of breastfeeding and efforts in reducing the protein content in infant and follow-on formula [3 4].

It remains unclear which child age period is most sensitive to a modified protein intake, and whether limiting protein intake during the second year of life would also achieve benefits for prevention of excessive weight gain and later obesity. Observational studies find a consistent association of later overweight and obesity with total protein intake and in particular of milk protein intake, not only during infancy but also during the preschool age [5-9]. A systematic review on the effects of dietary protein intake concluded that the first 2 years of life are the most sensitive time period [10].

The untoward programming effect of a high early protein intake on later obesity risk has been linked to its effects on increasing plasma and tissue concentrations of insulinogenic amino acids, insulin and insulin-like growth factor 1 (IGF-1), which appear to induce a higher weight gain during the first 2 years of life as well as an enhanced adipogenic activity [11]. Such effects of an infant formula higher protein content on insulinogenic amino acids, insulin and IGF-1 levels have been shown in the double-blind randomized CHOP trial [12-14].

Milk protein seems to play a key role in growth regulation during early childhood. Protein intake is the main contributor for nutritional regulation of the IGF-I axis [15 16]. Milk protein enhances serum IGF-1 to a greater extent than meat protein [17]. This might explain the more pronounced effect of milk protein compared to other proteins on the later risk of obesity that has been reported [8].

Average protein intake of young children in Europe and other regions is much higher than metabolic requirements. During the second year of life, 30-50% of total daily protein is comprised of dairy products [18 19], indicating particular opportunities to reduce overall protein consumption though modifying dairy protein intake.

Therefore, we designed a randomized controlled trial to examine the role of milk protein intake during the second year of life on child growth and later obesity risk. If a reduction of milk

protein during the second year of life has an appreciable effect on growth and obesity development, respective dietary modification may be translated into the practice of toddler feeding.

Main Objective

We aim at evaluating the effect of two iso-energetic milk products for young children with differing protein content on growth during the second year of life.

Secondary Study Objectives

Besides treating the study as an intervention study as described in detail below, the study incorporates a longer follow-up and is also considered a cohort study. Data obtained and produced should be scientifically exploited for explorative analysis specifically addressing the interplay and factors that influence child feeding, growth and development, physical activity, metabolism, and disease prevention.

Methods and analysis

Study design and population

The Toddler Milk Intervention trial (ToMI trial) is designed as a two-arm, parallel, randomized, double blind controlled trial to evaluate toddler milk products with different protein content. The study is conducted at university hospitals in Munich, Germany, and in Tarragona and Reus, Spain.

The target population are healthy children at the age of one year. The children are enrolled if they meet the inclusion and exclusion criteria outlined in Table1.

Intervention - formula composition

Two investigational formulas are used. Both formulas are based on cow's milk. The protein is unmodified from cow's milk and has the same casein:whey protein ratio in both formulas. The experimental formula contains 0.72g protein/100ml (1.5g/100 kcal), with a protein content that is similar to breast milk in advanced lactation. The control formula contains 2.95g protein/100ml (6.15g/100 kcal) which is comparable to standard 2% cows´ milk. Contents of energy, carbohydrates, vitamins and minerals are very similar for both formulas (Table 2). In order to reach the same energy content in both formulas, the fat content varies between

experimental (4.25g fat/100 kcal) and control formula (2.16g fat/100 kcal) but the lipid composition and the ratio of milk fat/vegetable oils is the same. Both formulas were developed and produced by the sponsor for this trial and were not tested in any other studies before the trial.

Dose, route of administration and schedule of formula

Participating families receive the formula as milk powder (one can comprises about 400g of product) and are advised to prepare the formula according to the instructions which were identical for all product codes. It is recommended to consume at least 300ml of formula per day. Further, parents are encouraged to substitute with the study formula any milk intake from the child´s diet. The intake of other dairy products such as cheese or yoghurt is accepted.

The intervention starts with the first study visit at around one year of age and ends with the third study visit at around two years of age. The study formula is given to the parents at no costs and is delivered directly to subject's home. Subject's compliance is regularly checked by telephone and personal interviews. After the end of the intervention, return and pick-up of remaining cans is organized. If not possible, families are advised to destroy remaining infant formula cans.

Discontinuation criteria

Discontinuation of the trial can be either due to withdrawal of consent at any time or due to the investigator´s decision that continuation within the trial might impair child´s health. All efforts will be undertaken to follow children irrespective of their study product consumption with all planned assessments.

Primary endpoint

The primary endpoint is BMI-for-age z-score (based on the WHO Multicentre Growth Reference Study [20]) at the age of 24 months adjusted for BMI-for-age z-score at 12 months of age.

Secondary objectives and endpoints

The secondary objectives serve to evaluate the safety and efficacy of the two milk products used and to complement the primary endpoint. Secondary endpoints will also be adjusted for baseline measurements if available. Secondary endpoints are:

- BMI-for-age z-score at 72 months,
- The percentage of overweight and obese children at 24 months of age according to CDC definition: Overweight is at and above the 85th to less than 95th percentile and obese 95th percentile or greater
- The percentage of overweight and obese children at 72 months of age,
- Anthropometric measures (z-scores for weight, length and head, waist and arm circumference at 12, 18, 24, 48 and 72 months of age; hip circumference at 48 and 72 month of age),
- Subcutaneous fat distribution (from skinfold thickness at 24, 48 and 72 months of age),
- Total body fat and lean mass (from BodPod measurements at 24, 48 and 72 months of age),
- Blood pressure (48 and 72 month of age),
- Child development (24 and 48 months of age),
- Metabolic and endocrine markers (IGF-1, IGF-BP2, IGF-BP3, insulin, leptin, adiponectin, ghrelin, lipid profile and complete blood count at 12, 24 and 72 month of age),
- Serum albumin, urea, creatinine, amino acids at 12, 24, 72 months of age and ferritin and 25-OH-vitamin D (at 24 months of age),
- Metabolic profile (from plasma at 12, 24 and 72 months of age and from urine samples at 12, 18, 24, 48 and 72 months of age),
- Urine markers (Calcium, C-peptide, creatinine urea nitrogen at 12, 18, 24, 48 and 72 months of age),

Furthermore, the following hypotheses will be examined:

- Total energy intake is not affected by the low protein formula.
- Total protein intake is lower in the group of protein reduced formula.
- Plasma concentrations of essential amino acids and of IGF-1 at the age of 24 months are lower in the low protein formula group compared to the high protein formula group.
- Systolic and diastolic blood pressure measurements at the ages of 48 and 72 months are lower in the low protein formula group compared to the high protein formula group.
- Body fat mass at age 24 months is lower in the low protein formula group compared to the high protein formula group.

- DNA methylation affects the association of protein intake and BMI
- Protein intake affects DNA methylation
- DNA methylation affects the association of protein intake and the metabolic profile

DNA methylation is currently only planned as an option provided additional funding can be secured.

Sample size

The sample size calculation is based on the observations from the CHOP-study [1]. This trial examined the difference in BMI-for-age z-scores between two groups of children fed a higher or lower protein content formula during the first year of life. At 24 months of age the BMI for age z-score difference between both formula groups was 0.2 standard deviations (SD). The absolute difference in protein content between intervention and control group in the CHOP-trial was lower (Infant formula: 0.8g/100ml; Follow-on formula: 1.6g/100ml) compared to the ToMI-trial (2.2g/100ml). Despite a higher protein difference, we expect a lower effect of the intervention due to the lower contribution of milk to the total protein intake in the second year of life. Thus, we assume a slightly lower mean difference in BMI for age z-score of 0.15 SD at 24 months of life.

The sample size was calculated with an anticipated effect size on BMI for age z-score of 0.15 SD and a standard deviation of 0.9. Assuming a power of 80 % and a significance level of 5% (two-sided alpha of 0.05), a sample size of 566 subjects per intervention arm is calculated. Therefore, 1,132 subjects in total are needed. To have enough power to detect also a difference of the same magnitude at 72 months (6 years) of age, at an assumed loss to follow-up of 30%, a final sample size of 1,618 subjects was estimated.

Recruitment

The study sites in Munich, Reus and Tarragona followed somewhat different recruitment strategies due to different local conditions. In Germany all inhabitants are registered in central registries. The public registries provided the study team for this defined research on a regular basis addresses of all families with children in the required age group (about 26,000 per year). These families living in Munich and about 70 surrounding municipalities were contacted once by postal mail and invited to contact the study team if interested in participation in the trial.

In Spain two recruitment strategies were used for both sites covering about 3000 births per year. First, telephone contacts from families who delivered their child at either of the two hospitals were available. These families were contacted directly. Second, recruitment interviews at primary health care centers were conducted. In these primary health care centers, Spanish children are seen for health care examinations and for vaccinations.

Allocation of study formula and blinding

The study formula cans are labelled with one of eight codes. Four codes each are assigned to the intervention or the control group, respectively. The allocation of the codes is performed online by study staff after check of in- and exclusion criteria within the data capture tool (iMedidata, Medidata Balance, New York, USA) using balanced randomization stratified by country. After enrolment of the subject into the trial, study staff dispense the assigned study formula to the study participant along with instructions for formula preparation.

The study is double blinded with all persons involved in local organization and conduct of the study such as study staff, principal investigator, project manager, biostatistician, data manager, trial monitor and laboratory analysts being unaware of the code allocation. After the code break for the primary outcome analysis, subjects will receive a new identification id in the analysis data to hamper the unblinding for above persons in the further follow-up. An emergency code break by an Investigator may be requested only in case of an unexpected serious adverse event (SAE) suspected to be related to the investigational product.

Data collection and management

During the intervention period three visits at the hospital are scheduled at 12, 18 and 24 months of age (Figure 1). At baseline socioeconomic data and data on health, growth and nutrition by 24-hours recalls during the first year of life are assessed. At each visit anthropometric measurements are performed and urine samples are collected. Blood is taken at 12 and 24 months of age. Additionally, at 24 months of age body composition using an air displacement plethysmography (BodPod COSMED, Rome, Italy) as well as physical activity measurement using an accelerometer device (Actigraph wGT3X-BT, Pensacola, FL, USA) is performed. Further, data of child´s development based on parent answers of the Ages & Stages questionnaire (ASQ-3, Brookes Publishing Co., Inc., USA) are collected.

For follow-up, two additional visits are scheduled at 48 and 72 months of age with anthropometric, body composition and physical activity measurements and collection of urine samples and food frequency questionnaires (Eating Habits Questionnaire -EHQ)[21]. Furthermore, socioeconomic data and data on health are updated and data on nutrition behavior is collected. At 48 months of age, the ASQ-3 is used again. Blood is taken at 72 months of age.

The main primary aim of the nutritional assessment during the intervention phase is to see if the intervention groups differ in nutritional intake. Therefore, a 24h-recall is used. While the second year of life is still considered a nutritional transition period, nutrition patterns are more stable between 48 and 72 months of age and analysis of food patterns are more relevant. Therefore, a FFQ is used for the later time points.

During all study visits and at several additional telephone calls between visits, parents are asked for health problems (including adverse events) and compliance. For compliance the intake of study milk and any discontinuation of study milk intake with reasons are determined. The number of consumed cans will be used to determine the average study milk consumption.

Collected data is organized in different databases. To organize and document all contacts with study participants and to coordinate the shipment of the study product, a web-based participant management tool is used (developed jointly with MedSciNet AB, Stockholm, Sweden). In this database, personal data is saved and stored on a secured data server. This database is separated from the other databases which store all medical, nutritional and laboratory data.

All collected health data are primarily captured on paper except data from questionnaires on physical activity and food frequency questionnaires that are entered by families using LimeSurvey (LimeSurvey GmbH, Hamburg, Germany). All other data are transferred from paper into web-based databases. Nutritional data from 24-hours recalls are entered into Nutritics (NUTRITICS LTD, Dublin, Ireland) with nutritional information from the German nutritional database BLS 3.02 and complemented with the nutritional composition from a variety of commercial infant foods and local foods, obtained directly from the label, producer websites or local food composition databases. All other data are entered into iMedidata (New York, USA).

Laboratory samples are processed according to a laboratory SOP. In general, aliquots have 2D barcodes, are scanned, linked with the subject ID and stored into 96-well racks at -80°C for later analysis. Only blood count, lipid status and HbA1c are measured locally on the day of blood sampling.

To ensure data quality, study staff is trained in regular intervals, and procedures are harmonized among study centers by regular contact and monitoring. Furthermore, anthropometric measurements are performed at least twice and data entry is strictly checked for consistency and plausibility by the monitor. Standard operating procedures for all measurements are in place; anthropometric measurements are based on the WHO Growth Standards study [20].

Statistical methods

A statistical analysis plan is created before final code break for the analysis of primary and secondary outcomes. For the statistical analysis, the full analysis dataset (FAS) and the per-protocol-dataset (PP) will be considered. The FAS comprises all randomized subjects who consumed at least one can of investigational product and was considered reasonable and as close as possible to the intention to treat (ITT) ideal as we dealt with a healthy population that participated not for treatment reasons. The PP compromises all subjects included in the FAS and that were compliant with the aimed product consumption (mean consumption of the recommended daily minimum amount of investigational product of 300ml/d). Compliance will be primarily assessed by the number of tins used by the study subject. A Blind Data Review Meeting with participants of the sponsor and the investigators will define specific rules and definitions for lack of compliance. No imputation of missing values is foreseen.

The primary endpoint will be analyzed in the FAS by linear regression (ANCOVA) and corrected for BMI-for-age z-score at baseline, study center and gender. The results of the final model will be compared to further adjusted models and analysis in the PP group; possible effect modification of the primary outcome will be also considered.

Secondary analyses supporting primary objective:

1. BMI-for-age z-score at 72 months.
2. The percentage of overweight and obese children at 24 months of age according to CDC definition: Overweight at and above the 85th to less than 95th percentile and obese 95th percentile or greater.

3. The percentage of overweight and obese children at 72 months of age.

In order to control the experiment wise false positive rate, the listed hierarchy (primary – secondary endpoints) will be maintained in interpreting these outcomes. The incidence of overweight and obese children at 24 and 72 months of age shall be also estimated according to International Obesity Task Force IOTF definition [22]. The percentage of overweight and obese children will be analyzed by the method of O. Sauzet, et al. [23].

Secondary endpoints include anthropometric measures, dietary and biochemical data. We will use z-scores of WHO growth standards for anthropometry measures at months 12, 18, 24, and 48. We will use a likelihood-ratio test to examine if there is a longitudinal treatment effect. Additionally, treatment differences at each visit will be analyzed using ANCOVA. The ANCOVA approach was chosen so that treatment differences and p-value do not depend on the stage of analysis. A further supportive analysis with a mixed linear model shall be performed at 6 years of age. Fixed effects shall be the intervention group, age, gender, and a two-way interaction between child age and intervention group will be included. The random effects shall be a random intercept and slope.

Dietary data is collected by 24-hours recalls or food frequency questionnaires, which allow us to test for differences in macronutrient intake using ANCOVA. Hence, we are able to analyze if subjects change their dietary habits over time.

Biochemical data is often log-normal distributed. In order to analyze this kind of data properly, we will log-transform the data to achieve approximately normal distributed residuals.

Interim Analysis

To ensure safety of the intervention, an interim analysis is planned when 260 subjects have completed the intervention (at 24 months of age). Non-inferiority for weight-for-age z-score has to be shown. This must be the case in both FAS and PP. A non-inferiority boundary for weight-for-age z-score of minus 0.5 SD was chosen according to Onyango et al. [24]. The same model as for the primary analysis is used. To demonstrate non-inferiority, the lower bound of the two-sided 95% confidence interval of the model based treatment difference must be larger than the non-inferiority margin.

If non-inferiority is shown, the study is continued as planned. Otherwise, a second stage interim analysis is performed including the first 390 subjects who have completed the intervention.

Furthermore, the safety evaluation will consider endpoints including adverse events, anthropometry, laboratory data and protein intake. Based on the results of the interim analysis and in accordance with the charter of the Data Monitoring Committee, the DMC will recommend either continuing the study as planned or performing the second stage interim analysis. The DMC is independent and consists of expert clinicians and statisticians with no competing interest. The planned interim safety analysis took place in June 2018 and no safety concerns were detected.

Besides the interim analysis, safety is continuously observed by blinded online monitoring of individual growth curves based on the WHO growth charts. If a considerable number of subjects drop below the median growth curve, an interim analysis will be initiated and the DMC will review unblinded data.

Harms

Any adverse events (AE) which lead to an untoward medical occurrence except for diagnostic and therapeutic non-invasive and invasive procedures will be recorded during the entire intervention period until 30 days after last study milk intake. After these 30 days, only AE´s which are related to the intervention treatment will be recorded. Each AE will be rated according to its severity and its relationship to the study milk. Additionally, severe adverse events (SAE) which e.g. requires inpatient hospitalization will be reported to the safety manager within 24 hours after notice and will be followed up until the outcome is known. A participant insurance is in place.

Monitoring

A commercial monitoring company reviews the process, AE reporting, data capturing and corresponding source data on a regular basis to ensure protocol compliance, accuracy and completeness.

Protocol versions

Issue date: 15.09.2020; version identifier: 5; number of protocol amendments: 5; initial version: 9 March 2016. First modification: 30 March 2016. Besides adaptation from requests of both ethical committees before the start of the study and several minor changes due to misspecifications in the protocol, several clarifications were needed, e.g. to provide more clarity and criteria for study termination before regular completion of the study, clarification in the

statistical interpretation of secondary endpoints, addition of new secondary endpoints physical activity and HbA1c, the adaptation to the new European data protection rules in 2018, and a change in exclusion criteria to allow the inclusion of children that are breastfed once per day. Furthermore, an extensive specification of the safety interim analysis after inclusion of 260 children was added in 2018 and more details for collection of AEs separating the collection into two periods, during and after the intervention, were provided.

Ethical considerations

This study is conducted in compliance with the International Conference on Harmonization (ICH) guidelines and the Declaration of Helsinki and complies with Good Clinical Practice guidelines. Ethics approval was obtained from the ethical committees of the university hospitals at the Ludwig-Maximilian University in Munich, Germany (Projekt Nr. 555-15) and at the Institut d'Investigació Sanitaria Pere Virgili, Reus, Spain (Ref. CEIm IISPV 013/2016. All protocol amendments were and will be approved by the ethical committee prior to implementation. All procedures and databases were approved by the local data protection agent and are in line with local and EU general data protection regulations.

Written informed consent is collected by study staff from all legal guardians prior to study inclusion in adherence with regulatory requirements with additional consent for genetic analysis. Each subject receives oral as well as written informed consent in plain language with adequate time in advance to make an informed decision about study participation. The latest informed consent form for both study sites is enclosed in the online supplementary (Supplementary file). All participants re-consented for any additional measurement added to the protocol.

Patient and Public Involvement

The study protocol was primarily developed at a public university hospital without involvement of the sponsor. There was no further public or patient involvement.

Public dissemination and data availability

Study results will be published in peer-reviewed journals and presented on national and international conferences. Study results will also be communicated to participants. Results will be written-up and published by the investigators without help of professional writers. Authorship will depend on relevant contribution to the study. Investigators have full research

freedom and have full access to all data. The full study protocol will be made available upon request. The participant-level dataset is not currently planned to be available because consent was not obtained for the sharing of such data from participant's parents / legal guardians or the Institutional Ethics Committees.

Trial status and time course of the trial

The study started to recruit subjects in September 2016 and finished recruitment of 1,625 children in October 2019. The intervention phase will last until October 2020. The database closure for the analysis of the primary outcome is planned for the first quarter of 2021. The follow-up will be completed around October 2025.

Funding, role of the sponsor and investigators

The sponsor has allocated a fixed budget for each study center to recruit and follow the subjects. The sponsor is producing the study product and distributes the study product to the study subjects. The sponsor is funding the monitoring of the study. The primary protocol was outlined by the investigators and was jointly further developed by investigators and sponsor. Data management will be primarily done by the sponsor, except parts of the compliance checks, checks of biosamples and body composition data, as well as nutritional and physical activity data. The primary analysis will be performed by the sponsor. The investigators have to approve the statistical analysis plan and will have full access to all the data. Any published interpretation of the data has to be in mutual agreement between sponsor and investigator without hampering the research freedom of the investigators. The urinary metabolic profile will be performed by the sponsor, all other laboratory measurements by the investigators. BK is the coordinating principal investigator with VG being his deputy, JE is principal investigator in Spain.

Authors´ Statement

VG and VJ wrote the manuscript. VG and BK provided the original outline of the protocol; JE, MZ, MG, and DG participated in the design and set-up of the study. BK, JE, MZ, MG, and DG critically revised the content of the original protocol and the manuscript.

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Conflict of interest

The institutions of VG, VJ, BK, JE, MZ, MG receive funding by the sponsor to conduct the study and DG is employed by the sponsor of the study.

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Tables

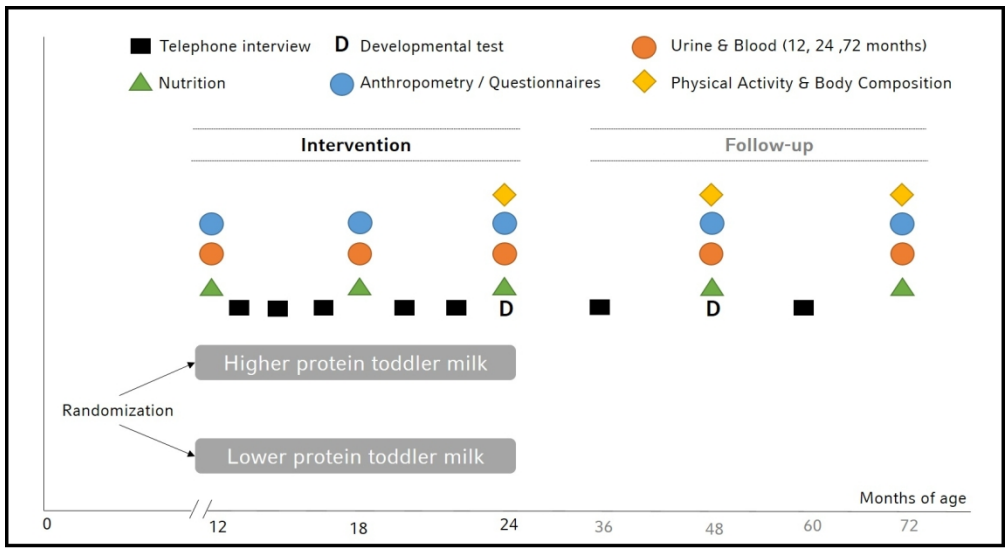
Table 1: Inclusion and Exclusion criteria of the Tomi trial

Inclusion criteria	Exclusion criteria
• Legal guardians signed the written informed consent.	• Infant who is breastfed at least twice in 24 hours at time of enrolment.
• Child was born full term (≥ 37 + 0 weeks of gestation).	• Infant who usually does not drink 300 ml of cow´s milk and/or formula milk per day.
• Child´s birth weight is between 2.5 and 4.5 kg.	• Cow´s milk allergy.
• Child is born from a singleton pregnancy.	• Lactose intolerance.
• Child´s age at enrolment is between 11.5 and 13.5 month.	• Institutionalized children.
• Child´s legal guardians are of legal age and they have sufficient local language skills to understand the study information, informed consent and study procedure.	• Diagnosed disorder, which interfere with nutrition or growth (e.g. celiac disease, inflammatory bowel disease).
• Child and child´s parents are willing to fulfil the requirements of the study protocol and procedures.	• Children who participated in any other interventional clinical trial 4 weeks prior to enrolment.
• Child´s family is available via phone or e-mail throughout the whole study.	

Table 2: Nutritional composition of the interventional products (toddler milks) that are based on cow's milk with the same casein:whey protein ratio.

	Experimental toddler milk (as prepared, per 100ml)	**Control toddler milk** (as prepared, per 100ml)
Energy	201 KJ/48 kcal	201 KJ/48 kcal
Protein	0.72 g	2.95 g
Fat	2.0 g	1.0 g
Saturated fatty acids	0.8 g	0.4 g
Carbohydrates	6.7 g	6.7 g
Lactose	6.7 g	6.6 g
Other	<0.1 g	<0.1 g
Salt	0.1 g	0.1 g
Vitamines		
Vitamine A	71 µg	66 µg
Vitamine D	1.2 µg	1.3 µg
Folic acid	14.9 µg	14.2 µg
Vitamine B12	0.2 µg	0.2 µg
Vitamine C	6.4 mg	6.9 mg
Minerals		
Calcium	115 mg	115 mg
Micronutrients		
Iron	0.5 mg	0.5 mg
Zinc	0.3 mg	0.6 mg

Figure 1: Assessments in children participating in the ToMI trial



Assessments in children participating in the ToMI trial

INFORMACIÓN A LOS PARTICIPANTES

TÍTULO	Efecto de la ingesta de proteínas lácteas en el niño pequeño sobre el crecimiento y el posterior riesgo de obesidad: ensayo clínico aleatorizado
ACRÓNIMO	**TOMI Trial**

INVESTIGADORES PRINCIPALES:
Ricardo Closa Monasterolo. Jefe del Servicio de Pediatría. Hospital Universitari de Tarragona Joan 23
Joaquín Escribano Subías. Jefe del Servicio de Pediatría. Hospital Universitari Sant Joan de Reus

INTRODUCCIÓN: Este documento es informativo sobre el proyecto de investigación que se indica en la cabecera, al cual les invitamos a participar. Les anticipamos que su participación es voluntaria y podrán realizar todas las preguntas que deseen, así como cambiar de opinión sobre su participación en cualquier momento. Su decisión no afectará la calidad de la atención sanitaria que reciba su hijo/a.

OBJETIVO: Este proyecto tiene como objetivo evaluar el efecto de dos fórmulas lácteas de crecimiento (con las mismas calorías, pero con diferente proporción de proteína y grasa) durante el segundo año de vida sobre el crecimiento desde el año hasta los 6 años.

INTERVENCIÓN NUTRICIONAL: Los niños/as de las familias que deseen participar recibirán de forma gratuita una de las dos leches de crecimiento del estudio durante todo el segundo año de vida (50% de probabilidad para cada una). Estas dos leches tendrán el mismo contenido energético (48 Kcal/100 ml) (calorías similares a la leche de vaca semidesnatada) y se diferenciarán en las proporciones de proteínas y grasas. Una de las leches tendrá 2.95g de proteínas y 1.1g de grasas (en 100ml), mientras que la otra tendrá 0.72g de proteínas y 2.11g de grasas (en 100ml). Estas proporciones se encuentran comprendidas entre las proporciones contenidas en la leche materna y la leche de vaca de consumo habitual. En ningún momento del estudio, ni los investigadores ni las familias conocerán cuál de estas leches consume cada participante.

METODOLOGÍA: En este estudio participaran unos 1618 niños de Múnich (Alemania) y Reus/Tarragona. La participación en el estudio tiene una duración de 5 años. Los participantes recibirán una de las dos leches de crecimiento desde el año hasta los 2 años de vida y se evaluará su crecimiento, desarrollo y estado nutricional y de salud a las siguientes edades: 1 año, 1.5 años, 2, 4 y 6 años (en total 5 visitas a lo largo de 5 años). La recogida de datos se llevará a cabo mediante las siguientes evaluaciones y procedimientos en diferentes momentos del seguimiento (que se detallan en la Tabla 1):

- Cuestionarios de salud completados por los padres (o persona a cargo del niño/a)
- Entrevistas telefónicas breves con el equipo de investigación (para revisar la alimentación)
- Exámenes (siempre voluntarios) realizados al niño/a, como:
  - Valoración del crecimiento y la composición corporal a través de medidas antropométricas.
  - Valoración de la composición corporal a través de desplazamiento de aire (se realiza sentado durante pocos minutos en una cámara cerrada llamada “BodPod”).
  - Tensión arterial (a los 4 y 6 años).
  - Actividad física a los 2, 4 y 6 años: la evaluación de la actividad física se realizará mediante cuestionarios específicos, completados por los padres (o persona a cargo del niño/a) y medida a

través de un monitor de actividad física o acelerómetro (Actigraph). El Actigraph es un monitor de actividad física (tipo acelerómetro) que consiste en un pequeño equipamiento médico (peso aproximado: 20gr) que se lleva en la cintura o cadera con un cinturón. Este equipamiento mide la actividad física, el sueño y el gasto energético. El procedimiento consiste en llevar el dispositivo unos 5-7 días para medir la actividad diurna (no hace falta llevarlo por la noche). Después, el dispositivo se retorna al personal del estudio para que extraigan de él los datos.

- Análisis de sangre: la extracción de sangre será realizada por personal cualificado a los 1, 2 y 6 años.
- Análisis de orina: los padres o cuidadores recogerán varias muestras de orina al participante a lo largo del estudio; esta recogida se efectuará mediante una bolsita para lactantes o mediante un tubo convencional de recogida de orina (material que les proporcionará de forma gratuita el equipo investigador) y se entregará en el momento de la visita.

CIRCUNSTANCIAS EN LAS CUALES LA PARTICIPACIÓN DEL SUJETO SE CONSIDERA FINALIZADA: En caso que el participante lo comunique o deje de acudir a las visitas. Mientras el participante no comunique su decisión de dejar de participar, el equipo de investigación seguirá invitándolo a asistir a las visitas. Asimismo, los participantes que no deseen continuar participando en el estudio o que no puedan seguir consumiendo el producto de estudio, serán invitados a acudir a una última visita a los 2 o 6 años.

EFECTOS ADVERSOS: Basados en investigaciones previas, no se espera ningún efecto indeseable por el consumo de la leche de estudio. En cualquier caso, dispondrán de teléfonos de contacto para notificar cualquier incidencia o realizarnos cualquier pregunta. Así mismo, si su hijo/a ha de ser ingresado/a en algún momento por cualquier motivo, rogamos nos lo hagan saber.

RIESGOS: El estudio no supone **ningún riesgo** que no sea el derivado de una extracción sanguínea. Las extracciones de sangre son analíticas normales, que realizará una enfermera con gran experiencia, y pueden causar las molestias propias de un pinchazo. La valoración del volumen corporal a través del desplazamiento de aire es una técnica totalmente segura que no provoca ninguna molestia. El uso del monitor para medir la actividad física no conlleva ningún riesgo. El dispositivo cumple con todos los requisitos de la Unión Europea por lo que respecta a dispositivos médicos de Clase I. En todo momento se tomarán precauciones para evitar al máximo cualquier inconveniente.
De todas formas, pueden seguir participando en el estudio, aunque decidan no realizar alguno de los exámenes anteriormente descritos.

BENEFICIOS: Aunque este proyecto no les promete ninguna ventaja directa, ustedes contribuirán a un mejor conocimiento de la importancia de la alimentación infantil sobre la obesidad infantil y el riesgo de padecer enfermedades cardiovasculares y posiblemente su participación servirá de ayuda a otras personas con estos problemas en el futuro.

DERECHOS DE LOS PARTICIPANTES

USO DE LAS MUESTRAS BIOLÓGICAS: Servirán para llevar a cabo determinaciones bioquímicas, metabólicas, epigenéticas y genéticas relacionadas con el objetivo del estudio (la obesidad y las enfermedades cardiovasculares). En primer lugar, se analizarán parámetros del estado nutricional general, los resultados de los cuales serán comunicados a las familias.
Una parte de las muestras de sangre y las muestras de orina serán enviadas anonimizadas a los laboratorios centrales del proyecto en Múnich (Labor für Stoffwechsel & Ernährung, Hauner Childrens Hospital y Laboratoriumsmedizin, KUM). Otras muestras codificadas pueden ser enviadas a Nestec, en Suiza, o a sus filiales o a terceros para hacer otros análisis. Usted puede decidir restringir el uso de estas muestras para

que no se lleven a cabo análisis genéticos (genes relacionados con la obesidad) indicándolo en la hoja de consentimiento.

Debido a la constante evolución del conocimiento y de las técnicas de investigación en esta área de la salud, es posible que en el futuro pueda realizarse una investigación complementaria relacionada con el objetivo del estudio. Por ello, los posibles sobrantes de las muestras de sangre y orina se preservarán en las mismas condiciones de anonimato y confidencialidad, y en un plazo máximo de 10 años serían destruidas. Ustedes pueden restringir la preservación de estas muestras indicándolo en la hoja de consentimiento. El tratamiento y uso de las muestras se realizará siguiendo lo especificado en la Ley de Investigación Biomédica (14/2007), y en el RD 1716/2011.

PROCEDIMIENTOS NO PLANIFICADOS: Debido a la constante evolución del conocimiento científico y las técnicas, el promotor y sus colaboradores pueden desarrollar análisis no planificados relacionados con los objetivos de este ensayo y/o relacionados con investigaciones futuras en el campo de la salud y/o nutrición. Si ustedes consintieran, las muestras biológicas sobrantes (sangre y orina) o los datos, serán almacenados bajo las mismas condiciones de anonimato y confidencialidad para poder ser reutilizadas en análisis complementarios y/o futuras investigaciones científicas (siempre relacionadas con la asociación entre la alimentación infantil, el crecimiento y la salud). Si ustedes reúsan, las muestras de su hijo/a serán almacenadas por un periodo máximo de 2 años y serán destruidas una vez el estudio y sus análisis estén terminados. Tienen el derecho de limitar el tiempo de retención y uso de estas muestras indicándolo en este consentimiento informado. Si aceptan el uso posterior de los datos y/o las muestras no planificadas en el protocolo inicialmente, serán informados y se les pedirá que den su consentimiento para estos análisis adicionales.

DEFINICIÓN DE DATOS PERSONALES: Datos personales son toda información que se relacione con una persona identificada o identificable. Una persona identificada o identificable es una persona natural que se puede identificar, directa o indirectamente, en particular a través de un identificador como por ejemplo un nombre o un código.

CONFIDENCIALIDAD: Para este estudio, las muestras biológicas obtenidas, así como toda la información recogida se codificarán con un número de forma que no aparezca ni su nombre ni su número de historia clínica. Únicamente los miembros del equipo de investigación tendrán acceso a sus datos y únicamente ellos podrían ponerse en contacto con ustedes y relacionar sus datos personales con los datos de salud recogidos. Para garantizar la calidad y seguridad del estudio, podrán supervisar la recogida de datos de salud: el monitor de calidad, las autoridades sanitarias, un representante autorizado de Nestlé y el Comité Ético de Investigación Clínica.

Se garantiza que todos los datos y resultados obtenidos serán **absolutamente confidenciale**s y que se utilizarán los mecanismos necesarios para el cumplimiento de la "Ley orgánica 15/1999, del 13 de Diciembre" para la protección de datos personales, y la "Ley 14/2007 de Investigación Biomédica ". El equipo de investigación de la *Unitat de Pediatria* de la *Facultat de Medicina de la Universitat Rovira i Virgili* será responsable de sus datos y muestras. El equipo de investigación garantiza su confidencialidad y el hecho que las muestras y los resultados sean utilizados únicamente para las finalidades consentidas. El responsable de sus datos personales codificados (estos datos no contienen ningún nombre o dirección suya o de su familia) es Nestec Ltd., con domicilio en Avenue Nestlé 55, CH-1800, Vevey, en Suiza. Los participantes tienen derecho a acceder, cambiar y oponerse al uso de sus datos, en cualquier momento, simplemente contactando con un investigador (derechos otorgados por Ley 15/1999). Tengan en cuenta que tienen además los derechos de ver y acceder a sus datos, de borrarlos, limitar su procesamiento o la transferencia, presentar una objeción al tratamiento en las circunstancias y los términos especificados en la normativa anterior (derecho concedido por la Ley 15/1999 y 18/2018 Coll., sobre protección de datos de

carácter personal y Reglamento UE 2016/679 del Parlamento Europeo y del Consejo de 27 de abril de 2016). No obstante, el promotor se reserva el derecho de no borrar los datos recogidos antes de retirar su consentimiento y que ya se hayan analizado como parte del estudio. Tienen el derecho de solicitar información sobre los datos del estudio recogidos por los doctores del mismo o por el promotor y sus afiliados (o representantes). Si desean ejercer estos derechos, o presentar una reclamación o solicitar la corrección de cualquier inexactitud de estos datos, pónganse en contacto con el médico del estudio o con el agente de protección de datos del Centro (*Unitat de Recerca en Pediatria i Desenvolupament Humà*. Sant Llorenç 21. 43201 Reus. Telf.977 759364 o 977 759365).

Si decidiesen retirar su consentimiento, solo deberán comunicarlo a los investigadores, en tal caso, no se incorporarán más datos a la base de datos y, si lo desean, también pueden solicitar por escrito la destrucción de sus muestras biológicas. Toda la información recogida en las visitas y exploraciones complementarias se codifica como el resto de muestras y datos del estudio TOMI con un número de forma que aparezca ni su nombre ni su número de historia clínica.

TRANSFERENCIA DE DATOS: Los datos del estudio recogidos de su hijo/a serán enviados al promotor, a terceros que trabajen para el promotor y a las autoridades reguladoras si así lo reclamaran. Solamente datos codificados se almacenarán mediante un sistema informático seguro que pertenece a Medidata, empresa ubicada en todo el mundo, un tercero de Nestlé. El acceso al sistema web está restringido al personal del estudio y a los representantes del promotor. El promotor también podrá utilizar los datos del estudio para poder comercializar la fórmula del ensayo en algunos países o para publicarlos. No obstante, nada que pueda revelar su identidad ni la de su hijo/a saldrá fuera del centro.

Sus datos codificados y los de su hijo/a serán transferidos y procesados a países distintos de España, en condiciones que garanticen su confidencialidad, desde el centro a Nestlé Suiza y otros países/organizaciones internacionales que actúen en nombre del promotor. Como responsable de los datos, Nestlé ha tomado medidas contractuales, organizativas y de seguridad que aseguren el mantenimiento del nivel de protección adecuado exigido por las leyes europeas y españolas, sea cual sea la tercera parte del estudio o los países a los que se transfieran los datos. Durante estos procedimientos no se divulgará su identidad ni la de su hijo/a.

VOLUNTARIEDAD: Su participación en este estudio es totalmente **voluntaria**; pueden decidir no participar, o cambiar su decisión y denegar su consentimiento en cualquier momento, hecho que no afectará ni perjudicará la relación con su médico ni su atención. Para ello, únicamente deberán comunicarlo al equipo de investigación.

INFORMACIÓN SOBRE EL ESTUDIO: Si se dispusiera de nueva información sobre el producto en estudio que pueda influir en su decisión de continuar en el mismo, se les informará de manera oportuna. En el caso de que estas investigaciones proporcionen datos que pudieran ser clínica o genéticamente relevantes para ustedes e interesar a su salud o a la de su familia, les serán comunicados salvo que indiquen expresamente que no desean recibir esta información. Aunque no deseen recibir esta información, tengan en cuenta que la ley establece que, cuando la información obtenida sea necesaria para evitar un grave perjuicio para la salud de sus familiares biológicos, un comité de expertos estudiará el caso y decidirá si es conveniente informar a los afectados o a sus representantes legales. Si por alguna razón ustedes quisieran conocer los resultados de las investigaciones que se hayan producido como consecuencia de su colaboración, podrán ponerse en contacto con los responsables del proyecto, que les informarán debidamente.

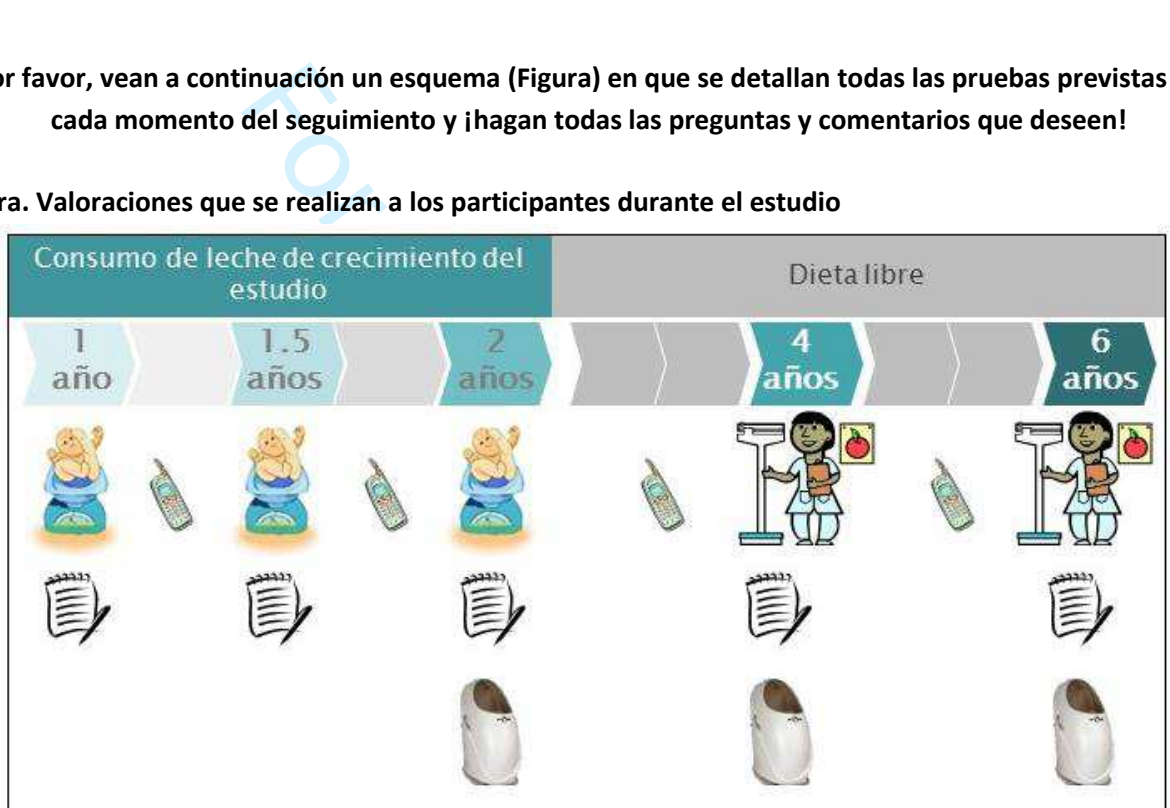
COMPENSACIÓN: Ustedes no recibirán incentivos económicos para participar en el estudio, pero recibirán una compensación que minimice el coste de tiempo y desplazamiento por acudir a la visita.

FONDO DE FINANCIACIÓN: Este estudio recibe soporte económico de Nestec Ltd., Avenue Nestlé 55 CH-1800 Vevey, Switzerland. Esta compañía es tomadora de un **seguro de responsabilidad** (contratado con la compañía Zurich Insurance plc., con nº de póliza Z140955 para el Hospital Universitari de Tarragona Joan XXIII y Z140963 para el Hospital Universitari Sant Joan de Reus) por cualquier posible consecuencia negativa sobre los participantes del estudio por su participación en el estudio. El promotor tiene la potestad de terminar el estudio en cualquier momento.

OTROS ASPECTOS REGULATORIOS: Este estudio ha sido aprobado por los Comités Éticos de Investigación Clínica del Institut d'Investigació Sanitària Pere Virgili y el de la Fundació Jordi Gol i Gorina. El estudio ha sido diseñado de acuerdo a la Declaración de Helsinki, que establece los criterios de investigación biomédica en personas de forma ética.

Por favor, vean a continuación un esquema (Figura) en que se detallan todas las pruebas previstas en cada momento del seguimiento y ¡hagan todas las preguntas y comentarios que deseen!

Figura. Valoraciones que se realizan a los participantes durante el estudio



INFORMACIÓN DE CONTACTO

Unitat de Pediatria, Facultat de Medicina. Universitat Rovira i Virgili. C/ Sant Llorenç 21, 43201 Reus.

Teléfonos: 977759365 / 977759364/ 619733840 (Tarragona)/ 616891314 (Reus)

(Copia para el participante)

CONSENTIMIENTO INFORMADO

Sr./Sra. ..... informa al padre/madre Sr./Sra. ..... en relación al estudio TOMI.

He sido informado/a sobre los derechos de mi hijo/a y los posibles riesgos y beneficios de su participación en el proyecto:

- La participación de mi hijo/a en este estudio es voluntaria.
- Se garantiza que los derechos y atención sanitaria de mi hijo/a no se verán influenciados por mi decisión.
- Los resultados relevantes que se obtengan a través de este proyecto podrán ser publicados, pero mis datos personales nunca serán revelados a no ser que lo requiera la ley.
- En el caso de que tenga cualquier duda o problema relacionado con el estudio, puedo contactar con Dr. Ricardo Closa, Dr. Joaquín Escribano, Dra. Natalia Ferré y Dra. Verónica Luque a través de los teléfonos 977759364, 977759365, 619733840 (Tarragona), 616891314 (Reus).
- Me ha sido entregada la hoja de información a los participantes y he sido informado/a de la naturaleza del estudio, que se resume en dicha hoja.
- He podido hacer preguntas para aclarar mis dudas.
- Puedo hacer cualquier pregunta en cualquier momento sobre este proyecto.
- He sido informado/a sobre mis derechos como participante en la investigación y, voluntariamente consiento en ceder mi material biológico y genético (muestras de sangre y orina) bajo las condiciones descritas y únicamente para los objetivos definidos.

Respondiendo a las preguntas de abajo declaro que:

- Deseo ser informado de los resultados clínicamente relevantes. Si □ No □
- Accedo a los análisis genéticos y epigenéticos opcionales que se realizaran dentro del marco de este estudio. Si □ No □
- Acepto que las muestras y datos sean utilizados y almacenados más tiempo que para los objetivos concretos del estudio y puedan ser utilizadas en futuros estudios científicos dentro del campo de la salud o la nutrición. Si □ No □
- Si la respuesta a la pregunta 3 es "No": Accedo a que se me contacte en un futuro en el caso de que estime oportuno añadir nuevos datos a los recogidos inicialmente o muestras biológicas adicionales. Si □ No □

Firma del padre/ tutor	**Firma de la madre/ tutor**	**Firma del informador**
Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _
Su firma indica que usted ha leído y entiende la información antedicha, que usted ha discutido este estudio con la persona que obtiene este consentimiento, que usted ha decidido participar basado en la información proporcionada, y que se le ha dado a usted una copia de este formulario. En caso que únicamente uno de los dos progenitores o cuidadores legales esté presente en esta entrevista, su firma implica que el otro progenitor está de acuerdo. De todas formas se le facilitará una hoja de firma para que esta sea entregada en la siguiente visita.		Declaro que los requisitos para el consentimiento informado para este proyecto han sido satisfechos, que he proporcionado al participante una copia de este formulario, discutido con él/ella el proyecto y le he explicado en términos no técnicos la información contenida en este documento. Igualmente, certifico que animé al participante a que hiciera preguntas y que todas fueron contestadas.

(Copia para el investigador) **CONSENTIMENTO INFORMADO** **ID**:_____

Sr./Sra. ……. informa al padre/madre Sr./Sra. ……..... en relación al estudio TOMI.

He sido informado/a sobre los derechos de mi hijo/a y los posibles riesgos y beneficios de su participación en el proyecto:

- La participación de mi hijo/a en este estudio es voluntaria.
- Se garantiza que los derechos y atención sanitaria de mi hijo/a no se verán influenciados por mi decisión.
- Los resultados relevantes que se obtengan a través de este proyecto podrán ser publicados pero mis datos personales nunca serán revelados a no ser que lo requiera la ley.
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- He podido hacer preguntas para aclarar mis dudas.
- Puedo hacer cualquier pregunta en cualquier momento sobre este proyecto.
- He sido informado sobre mis derechos como participante en la investigación y, voluntariamente consiento en ceder mi material biológico y genético (muestras de sangre y orina) bajo las condiciones descritas y únicamente para los objetivos definidos.

Respondiendo a las preguntas de abajo declaro que:

- Deseo ser informado de los resultados clínicamente relevantes. Si □ No □
- Accedo a los análisis genéticos y epigenéticos opcionales que se realizaran dentro del marco de este estudio. Si □ No □
- Acepto que las muestras y datos sean utilizados y almacenados más tiempo que para los objetivos concretos del estudio y puedan ser utilizadas en futuros estudios científicos dentro del campo de la salud o la nutrición. Si □ No □
- Si la respuesta a la pregunta 3 es "No": Accedo a que se me contacte en un futuro en el caso de que estime oportuno añadir nuevos datos a los recogidos inicialmente o muestras biológicas adicionales. Si □ No □

Firma del padre/ tutor	**Firma de la madre/ tutor**	**Firma del informador**
Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _	Fecha _ _ / _ _ / _ _ _ _
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(Copia para el investigador, en caso que se obtenga posteriormente el consentimiento de uno de los dos progenitores)

CONSENTIMENTO INFORMADO **ID**:_____

Sr./Sra. ……. informa al padre/madre Sr./Sra. ……. en relación al estudio TOMI.

He sido informado/a sobre los derechos de mi hijo/a y los posibles riesgos y beneficios de su participación en el proyecto:

- La participación de mi hijo/a en este estudio es voluntaria.
- Se garantiza que los derechos y atención sanitaria de mi hijo/a no se verán influenciados por mi decisión.
- Los resultados relevantes que se obtengan a través de este proyecto podrán ser publicados pero mis datos personales nunca serán revelados a no ser que lo requiera la ley.
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- Puedo hacer cualquier pregunta en cualquier momento sobre este proyecto.
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- Accedo a los análisis genéticos y epigenéticos opcionales que se realizaran dentro del marco de este estudio. Si □ No □
- Acepto que las muestras y datos sean utilizados y almacenados más tiempo que para los objetivos concretos del estudio y puedan ser utilizadas en futuros estudios científicos dentro del campo de la salud o la nutrición. Si □ No □
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Toddler Milk Intervention Study

Elterninformation und Einverständniserklärung

Einfluss von Milcheiweiß auf das Wachstum und die spätere Entwicklung von Übergewicht (ToMI-Studie)

Studienregistrierung: NCT 02907502 bei clinicaltrials.gov

Bitte lesen Sie diese Information und Einverständniserklärung sorgfältig durch. Das Studienpersonal wird Ihnen jederzeit alle Fragen beantworten.

Die ToMI-Studie wurde durch die Ethikkommission und den Datenschutzbeauftragten des Klinikum der Universität München geprüft und zustimmend bewertet.

Sie erhalten eine Kopie dieses Schreibens für Ihre Unterlagen.

Liebe Familie,

wir am Dr. von Haunerschen Kinderspital in München führen eine Studie zum Einfluss von Milcheiweiß auf Gewicht und Wachstum von Kindern durch. Die Studie heißt ToMI-Studie (ToMI von engl. ***to****ddler's* ***m****ilk* ***i****ntervention* = Kleinkindermilch Intervention).

Warum führen wir die Studie durch

Die zunehmende Häufigkeit von Übergewicht und Fettleibigkeit (Adipositas) stellt ein großes medizinisches Problem dar. Inzwischen sind alle Altersgruppen davon betroffen, insbesondere auch Klein- und Schulkinder. Wir befassen uns sehr intensiv mit den frühkindlichen Ursachen für diese Entwicklung. Unter anderem leiten wir das weltweit größte Forschungsprojekt zu Auswirkungen der frühkindlichen Ernährung auf die Gesundheit im späteren Leben (http://www.project-earlynutrition.eu). Vor einigen Jahren konnten wir in einer anderen EU-finanzierten Studie („CHOP-Studie") mit Säuglingen zeigen, dass ein niedrigerer Eiweißgehalt in der Säuglingsnahrung während des ersten Lebensjahres dazu beiträgt, dass die Kinder im Schulalter seltener übergewichtig sind.

Bei der ToMI-Studie soll nun untersucht werden, ob sich die gleiche Wirkung durch weniger Milcheiweiß auch im zweiten Lebensjahr zeigt. Dafür wurde speziell eine Kleinkindermilch mit reduziertem Eiweißgehalt hergestellt, die im Vergleich zu herkömmlicher Kleinkindermilch und Kuhmilch deutlich weniger Milcheiweiß enthält.

Neben der Ernährung ist auch das Maß an körperlicher Aktivität in der Kindheit ausschlaggebend für die gesunde Entwicklung eines Kindes. Wir wollen dabei vor allem den Zusammenhang zwischen der frühen Ernährung und dem kindlichen Aktivitätsverhalten untersuchen, aber auch mehr über mögliche Einflussgrößen für das Aktivitätsniveau Ihres Kindes herausfinden.

Studienzweck

Ziel der ToMI-Studie ist es, das Wachstum, die Entwicklung und den Stoffwechsel von Kleinkindern zu untersuchen, die im zweiten Lebensjahr eine eiweißreduzierte Kleinkindermilch erhalten.

Ablauf der Studie (siehe auch Bild 1)

Falls Sie der Teilnahme zustimmen, wird Ihr Kind zufällig entweder der herkömmlichen oder einer eiweißreduzierten Kleinkindermilch zugeteilt. Um die Studienergebnisse nicht beeinflussen zu können, werden weder Sie noch wir erfahren, welche Kindermilch Ihr Kind bekommt. Die Studienmilch soll im 2. Lebensjahr alle anderen Milchgetränke und -nahrungen, somit auch Kuhmilch, ersetzen. Sie erhalten die Studienmilch von uns kostenfrei für das gesamte zweite Lebensjahr. Mit dem zweiten Geburtstag Ihres Kindes endet die Phase, in der Ihr Kind die Studiennahrung bekommt. Insgesamt werden 1618 Kleinkinder an der ToMI-Studie teilnehmen (davon 809 in München und 809 in Reus und Tarragona in Spanien) und vom 1. bis zum 6. (72. Monat) Geburtstag beobachtet.

Im Alter von 12, 18, 24, 48 und 72 Monaten werden wir Ihr Kind im Dr. von Haunerschen Kinderspital sehen. Bei jedem Besuch werden wir Ihr Kind untersuchen und Größe, Gewicht und weitere Körpermaße aufnehmen. Wir werden Ihnen jeweils

Fragen zur Gesundheit und Verhalten Ihres Kindes stellen. Um zu erfahren, wie und wo Ihr Kind aufwächst, werden wir Sie anfangs auch zu Ihrer Herkunft, Ausbildung und Familienstruktur sowie zu Ernährungsgewohnheiten im ersten Lebensjahr befragen. Um zu verstehen wie sich Ihr Kind sonst ernährt, werden wir Sie zu jedem Zeitpunkt fragen, was und wieviel Ihr Kind in den vergangenen 24 Stunden gegessen und getrunken hat. Den Urin Ihres Kindes würden wir gerne jedes Mal untersuchen.

Im Alter von 24 und 48 Monat bitten wir Sie einen Fragebogen zur allgemeinen Entwicklung Ihres Kindes auszufüllen. Ab dem 2. Lebensjahr bestimmen wir die Körperzusammensetzung mittels BodPod®. Die BodPod®-Messung ist eine kurze, unkomplizierte Untersuchung mittels Luftverdrängung zur Bestimmung des Körperfettanteils (http://www.bodpod.com/de/produkte/koerperzusammensetzung).

Im Zuge der Studienbesuche mit 2, 4 und 6 Jahren wollen wir die körperliche Aktivität Ihres Kindes messen. Zusätzlich möchten wir mit Hilfe eines Fragebogens Daten über die körperliche Aktivität von Ihnen und Ihrem Kind sammeln. Die Aktivität wird mit einem Akzelerometer (wGTX3-BT, ActiGraph, Pensacola, USA) gemessen. Der Sensor wird mit Hilfe eines Gummibandes an der Hüfte Ihres Kindes befestigt. Aus den gewonnenen Daten können wir Rückschlüsse auf die tägliche Dauer und Intensität des Bewegungsverhaltens Ihres Kindes ziehen.

Eine Blutabnahme (ca. 6 ml) ist am Anfang und mit 2 und 6 Jahren vorgesehen. Wenn es gewünscht wird, können wir zuvor etwas Emla® Crème auf die Haut Ihres Kindes auftragen, um die Einstichstelle örtlich zu betäuben.

Wir werden Sie zusätzlich alle 2-6 Monate kontaktieren, Sie anfangs zum Verzehr der Studiennahrung befragen und uns kurz nach dem Wohlbefinden Ihres Kindes erkundigen.

Weitere Informationen zur Studie finden Sie auch auf unserer Homepage unter http://www.klinikum.uni-muenchen.de/de/forschung/TOMI-Studie.html.

Eine Beschreibung der Studie steht auch unter http://www.clinicaltrials.gov zur Verfügung.

Die Studiennahrung wurde von der Firma Nestec (Avenue Nestlé 55, CH - 1800 Vevey, Schweiz) für die Studie entwickelt und produziert. Die Nahrung entspricht den europäischen Richtlinien und industriellen Standards. Sie enthält 48 kcal / 100ml Energie und 0,7 g / 100ml bzw. 3,0 g / 100ml Eiweiß in der Eiweiß-reduzierten bzw. der herkömmlichen Kindermilch. Sie ist geeignet für die Ernährung von Kleinkindern im Alter von 12 bis 24 Lebensmonaten und darf nur in diesem Zeitraum durch das Studienkind konsumiert werden.

<u>Familienkost, Beikost und Getränke</u>

Natürlich darf Ihr Kind auch während der Studie seine gewohnte Kleinkinderkost bzw. Familienkost zu sich nehmen. Wir bitten Sie nur, die Milchmahlzeiten Ihres Kindes durch Studiennahrung zu ersetzen. Auch die Herstellung von Breimahlzeiten, Puddings oder ähnlicher milchhaltiger Speisen soll möglichst mit der Studienmilch erfolgen. Nach dem 2. Geburtstag sind Sie völlig frei bei der Ernährung Ihres Kindes.

<u>Nutzen und Risiken bei der Teilnahme an der Studie</u>

Durch die Teilnahme an dieser Studie bekommt Ihr Kind die Möglichkeit, eine neuartige Kleinkindermilch zu verzehren. Die Kleinkindermilch wird nach europäischen Richtlinien und industriellem Standard hergestellt. Die neuartige Kleinkindermilch enthält ausreichend Eiweiß und ist im Eiweißgehalt vergleichbar mit Muttermilch. Trotzdem kann es zu Unverträglichkeiten bei Ihrem Kind kommen. Wir erwarten jedoch keine Reaktionen, die über das normale Maß bei Verwendung von Kleinkindermilch hinausgehen.

Eine Teilnahme an der Aktivitätsmessung kann wichtige Hinweise auf das Aktivitätsverhalten Ihres Kindes liefern. Sie erhalten nach der Abgabe des Akzelerometers eine individuelle Einschätzung, welche Ihnen hilft, das Aktivitätsniveau Ihres Kindes besser zu verstehen und ggf. gezielt zu fördern.

Auch wenn das Gerät sehr robust ist und in der alltäglichen Nutzung nicht beschädigt werden kann, ist jedoch bei grober Gewalt die Ablösung von Kleinteilen möglich, die verschluckt werden können.

Das Risiko bei der Blutentnahme ist verschwindend gering. Es ist möglich, dass es zur Bildung eines blauen Flecks und in den seltensten Fällen zu Infektionen an der Einstichstelle kommt.

Falls im Verlauf der Studie wichtige neue Erkenntnisse bekannt werden, die sich auf Ihre Entscheidung über die weitere Teilnahme an dieser Studie auswirken könnten, werden Sie darüber umgehend informiert. Sie erhalten ggfs. eine neue Elterninformation und Einverständniserklärung zum Unterzeichnen, sofern Sie weiter an der Studie teilnehmen möchten.

Sie können aus der Studie ausgeschlossen werden, wenn es medizinische oder organisatorische Gründe notwendig machen. In diesem Falle werden wir Sie darüber informieren und die bis dahin erhobenen Daten anonymisiert verwenden.

Laboruntersuchungen

Blutwerte liefern wichtige Informationen, um die Auswirkungen der Ernährung auf den Stoffwechsel des Körpers beurteilen zu können. Entscheidend sind für uns aber nicht die einzelnen Werte Ihres Kindes – wie bei Krankheiten oder der Bewertungen durch Ihren Kinderarzt -, sondern der Mittelwert von allen ToMI-Kindern. Das bedeutet: Es sollten möglichst alle Kinder mitmachen, damit wir tatsächlich neue Erkenntnisse aus dem Blut Ihres Kindes gewinnen können! Daher hoffen wir sehr, dass Sie einer Blutentnahme bei Ihrem Kind zustimmen. In den Blut und Urinproben führen wir neben Routineuntersuchungen zur Gesundheit (z.B. Blutbild) vor allem Messungen von Stoffen durch, die mit der Eiweiß- und Energieverwertung (z.B. Harnstoff, Glukose, Blutfette) zusammenhängen. Daneben werden Hormone, die mit Wachstum und Gewichtsentwicklung im Zusammenhang stehen, bestimmt. Wir werden Sie über das Blutbild sowie die Untersuchung von Blutfetten informieren. Alle anderen Blutwerte werden erst am Ende der Studie bestimmt und dienen ausschließlich wissenschaftlichen Zwecken.

Um die Proben zu verschlüsseln, werden sie statt mit dem Namen Ihres Kindes mit einem „Pseudonym" versehen. Das Pseudonym ist eine Kombination aus Buchstaben und Zahlen. Nur mit Hilfe von Computerprogrammen (Pseudonymisierungsschlüssel), die Kind und Pseudonym einander zuordnen, kann herausgefunden werden, welche

Probe zu welchem Kind gehört. Der Pseudonymisierungsschlüssel wird nicht an Dritte weitergegeben.

Da in der Forschung ständig neue Erkenntnisse gewonnen werden, bitten wir Sie um die Erlaubnis, eventuell überschüssige Blutproben anonymisiert (eine Zuordnung zu Ihrem Kind ist nicht mehr möglich) bis zu 10 Jahre nach Studienende aufbewahren zu dürfen, damit Blut nicht vergeudet und noch für künftige, innovative Analysen zur Verfügung steht.

Genetische Untersuchungen

Eine Frage die uns beschäftigt ist, wie Veränderungen am Anfang des Lebens (in dieser Studie eine Veränderung der Ernährung im 2. Lebensjahr) den Stoffwechsel und die Gesundheit später beeinflussen können. Eine Möglichkeit, warum es zu einer langfristigen, eventuell lebenslangen Prägung kommen könnte, sind Veränderungen in der Steuerung der Genaktivierung. Während man vor kurzem noch glaubte, dass man Erbfaktoren, also Gene, einfach hat oder nicht hat, weiß man heute viel mehr, wie Gene „an- und ausgeschaltet" werden können („Epigenetik"). Durch eine Untersuchung der Erbsubstanz im Blut können wir feststellen, welche für den Stoffwechsel, die Körperzusammensetzung, Übergewicht und damit einhergehende Erkrankungen relevante Gene an- oder ausgeschaltet wurden.

Wenn Sie der Untersuchung zustimmen, wird aus einer Blutprobe Ihres Kindes die Erbsubstanz (DNA) gewonnen und untersucht. Die Blutproben werden im Alter von 12, 24 und 72 Monaten gesammelt, um Veränderungen in der Steuerung der Gene feststellen zu können. Die eigentlichen genetischen Untersuchungen erfolgen erst zu einem späteren Zeitpunkt, wenn von möglichst allen Probanden die DNA zu den drei genannten Zeitpunkten gewonnen wurde.

Für die Genuntersuchung muss keine zusätzliche Blutprobe abgenommen werden. Es wird das „Abfallprodukt" der übrigen Blutproben verwendet, die abgetrennten Blutzellen, die ansonsten für keine Untersuchung genutzt werden können. Aus diesen Zellen wird die Erbsubstanz (DNA) gewonnen und die meisten der bisher bekannten, informationsenthaltenden Abschnitte des Erbguts untersucht. Anhand dieser Informationen können wir feststellen, welche Gene an- und ausgeschaltet wurden, die für Stoffwechsel, Körperzusammensetzung und Übergewicht sowie die assoziierte Erkrankungen relevant sind. Außerdem können wir diese Veränderungen in Zusammenhang mit den vielen Einflüssen betrachten, die wir im Rahmen der Studie bei Ihrem Kind beobachten.

Aus der Untersuchung von Erbfaktoren und deren Aktivität ergibt sich für Ihr Kind kein direkter Vorteil. Mit Ihrer Teilnahme unterstützen Sie jedoch die Forschung, wie frühkindliche Ernährung und Verhaltensweisen sowie Umweltfaktoren andauernde Veränderungen verursachen. Dadurch kann möglicherweise die Grundlage für Verbesserungen in der Diagnose und Behandlung von Erkrankungen gelegt werden.

Die Untersuchungen auf Erbfaktoren werden pseudonymisiert bzw. in irreversibel anonymisierter Form am Helmholtz-Zentrum München, Institut für Molekulare Epidemiologie durchgeführt. Durch eine doppelte Kodierung (den pseudonymisierten Proben wird vor der Aufarbeitung eine fortlaufende Labor-Nummer zugeordnet) ist es den Mitarbeitern des Helmholtz-Zentrums nicht möglich, Rückschlüsse auf die persönlichen Daten des Probanden zu ziehen. Damit ist sichergestellt, dass diese

besonders sensiblen genetischen Daten zusätzlich geschützt werden. Die genetischen Untersuchungen werden nur für Forschungszwecke im Rahmen der ToMI-Studie durchgeführt. Es ist nicht möglich und nicht vorgesehen Ergebnisse mitzuteilen. Die statistische Auswertung der genetischen Daten wird unter Verantwortung von Prof. B. Koletzko durchgeführt, ohne Bezug zum Namen Ihres Kindes.

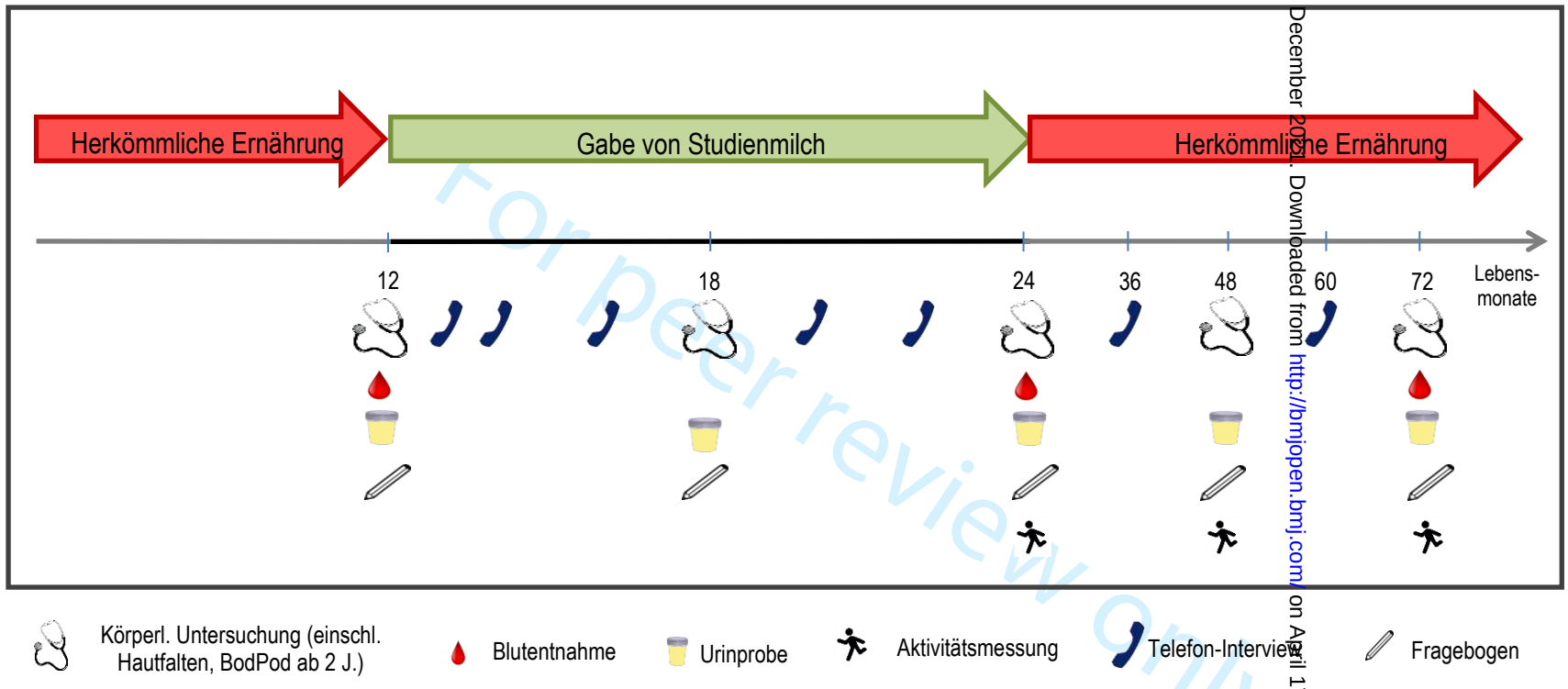

Bild 1. Ablauf der Studie

Studienauswertung

Die Daten, Proben und Fragebögen werden ausschließlich für den oben genannten Studienzweck verwendet. Die Studienauswertung wird gemeinsam mit Nestec durchgeführt. Die Veröffentlichung von Ergebnissen und deren Interpretation erfolgt einvernehmlich.

Studienfinanzierung

Die Studie wird durch die Firma Nestec Ltd. (Avenue Nestlé 55, CH - 1800 Vevey) finanziert. Die Finanzierung umfasst das nötige Studienpersonal, Laboruntersuchungen und die Studiennahrung. Weitere wissenschaftliche Untersuchungen werden durch öffentliche und gegebenenfalls private Finanzierungen erfolgen.

Versicherungsschutz

Auch wenn keinerlei Komplikationen erwartet werden, so sind doch alle Studienteilnehmer durch eine Studienversicherung abgesichert. Der Versicherungsschutz erstreckt sich auf alle Gesundheitsschädigungen, die als Folge der im Zusammenhang mit der Studie angewendeten Maßnahmen eintreten bis zu einer Höchstsumme von € 5.000.000.
Im Schadensfalle können Sie sich direkt an den Versicherer (Zurich Insurance plc NfD, Solmsstraße 27-37, 60486 Frankfurt am Main, Tel.: 069 7115-0; Policen-Nummer: 801.380.024.996) wenden und Ihre Ansprüche geltend machen. Um den Versicherungsschutz nicht zu gefährden, müssen Sie folgendes beachten:

- Teilen Sie uns alle medizinischen Behandlungen mit, denen sich Ihr Kind während der Studienphase unterzieht (Ausnahmen sind Vorsorgeuntersuchungen und Impfungen). Dies gilt auch für die Einnahme neuer Medikamente.
- Teilen Sie eine Gesundheitsschädigung, die als Folge der Studienteilnahme eingetreten sein könnte, bitte dem zuständigen Studienpersonal und der oben genannten Versicherungsgesellschaft mit.

Freiwilligkeit / Rücktrittsklausel

Die Teilnahme an der Studie ist freiwillig. Mit Ihrer Einwilligung auf der „Einverständniserklärung" geben Sie Ihr Einverständnis zur Teilnahme Ihres Kindes an dieser Studie. **Sie haben das Recht, zu jeder Zeit ohne Angabe von Gründen und ohne Nachteile die Teilnahme an der Studie zu beenden**.

Aufwandentschädigung

Für die Teilnahme an der Studie erhalten Sie eine Aufwandsentschädigung.

Wenn Sie weitere Fragen zu dieser Studie haben oder wenn Sie der Ansicht sind, eine studienbezogene Gesundheitsschädigung erlitten zu haben, stehen wir Ihnen gern zur Verfügung: Dr. V. Grote, V.Jäger, M. Meier, S. Vogt, N. Antl, und P. Becker.
Tel:089-4400-57427; E-Mail: Tomi.Studie@med.uni-muenchen.de

Datenschutz: Im Rahmen der Studie gelten folgende Regeln des Datenschutzes.

Datenschutz

Bei dieser Studie werden die Vorschriften über die ärztliche Schweigepflicht und den Datenschutz entsprechend den europäischen, deutschen und bayerischen Richtlinien und der Deklaration von Helsinki eingehalten. Um Sie kontaktieren zu können, werden Ihre Kontaktdaten in einer Datenbank (MedSciNet, Stockholm, Schweden, http://medscinet.com/) gespeichert. In dieser Datenbank werden persönliche, jedoch keinerlei medizinischen Daten gespeichert. Zur Auslieferung der Studiennahrung erfolgt eine Weitergabe Ihrer Adressdaten an ein externes Logistik-Unternehmen (OCasa Lodilat Logistica S.L., Avda de la Astronomia 8, 28830 San Fernado de Henares, Spain). Eine Weiterverwendung dieser Daten zu anderen Zwecken als der Auslieferung der Studiennahrung ist dem Unternehmen untersagt. Das Unternehmen unterliegt den deutschen gesetzlichen Datenschutzbestimmungen.

Alle weiteren Daten – also „medizinische Daten" -, die nicht der Kontaktaufnahme und Kontaktorganisation dienen, werden in getrennten Datenbanken (Medidata Solutions, 350 Hudson St, New York, NY 10014 sowie lokal im Klinikum der Universität München)gespeichert. Persönliche Daten wie Name oder Adresse werden in diesen Datenbanken nicht erfasst. Die Zuordnung zum Namen Ihres Kindes kann nur über einen Verschlüsselungscode erfolgen, der nur unter aktiver Hilfe des Studienpersonals einem Namen zugeordnet werden kann. So sind alle erhobenen Daten und Befunde Ihres Kindes pseudonymisiert.

Sie haben das Recht, jederzeit Auskunft über Ihre gespeicherten personenbezogenen Daten zu erhalten, diese zu berichtigen oder ggf. löschen zu lassen. Verantwortlich für die Datenverarbeitung ist Prof Dr. Berthold Koletzko sowie Dr. Veit Grote als dessen Stellvertreter.

Kontaktdaten der Datenschutzbeauftragten:

Bei Beschwerden haben Sie das Recht sich an die jeweilige Datenschutz-Aufsichtsbehörde zu wenden.

Der lokale Datenschutzbeauftragte für das Klinikum der Universität München ist:

Herr Gerhard Meyer
Klinikum der Universität München
Pettenkoferstr. 8
80336 München
E-Mail: datenschutz@med.uni-muenchen.de

Die übergeordnete Behörde für die LMU und das Klinikum ist:

Bayerischer Landesbeauftragter für den Datenschutz (BayLfD)
Postanschrift: Postfach 22 12 19, 80502 München
Hausanschrift: Wagmüllerstr. 18, 80538 München
Tel.: 089 212672-0
Fax: 089 212672-50

Datenzugang:

Der Zugang zu den Adressdaten und zum Verschlüsselungscode ist auf folgende Personen der Studienorganisation beschränkt: Prof. B. Koletzko, Dr. V. Grote, V. Jäger, M. Meier, S. Vogt, N. Antl, P. Becker und U. Handel. Weitere Personen aus dem Studienzentrum (Dr. von Haunersches Kinderspital, Abt. Stoffwechsel und Ernährungsmedizin unter der Leitung von Prof. B. Koletzko) können zur Studienorganisation im Verlauf der Studie nach Zustimmung der Studienleitung Zugang erhalten. Die Firma Nestec hat darüber hinaus die Firma PAREXEL International GmbH beauftragt, die Qualität der Studie vor Ort zu überwachen (sog. „Monitoring"). Das Unternehmen wird zum Datenschutz verpflichtet und hat vor Ort Zugang zu persönlichen und medizinischen Daten. Eine Entschlüsselung einzelner Studienteilnehmer erfolgt lediglich in Fällen, in denen es die Sicherheit erfordert („medizinische Gründe"). Das Unternehmen unterliegt den deutschen, gesetzlichen Datenschutzbestimmungen.

Die Firma Nestec hat kontinuierlichen Zugang zu pseudonymisierten Daten, jedoch nie zu den Kontaktdaten. Diese pseudonymisierten Daten werden von Nestec auch in anderen Ländern als Deutschland oder der Schweiz (Sitz von Nestec) verarbeitet. Hierbei wird Ihre Identität gewahrt und die Vertraulichkeit Ihrer Daten gewährleistet. Es gelten für diese Drittländer /internationale Organisationen vertraglich die europäischen und deutschen gesetzlichen Datenschutzbestimmungen.

Einige Stoffwechseluntersuchungen werden in den Laboratorien der Firma Nestec, Avenue Nestlé 55, CH - 1800 Vevey, Schweiz durchgeführt. Die genetischen und epigenetischen Analysen werden in Zusammenarbeit mit dem Helmholtz-Zentrum, Institut für Molekulare Epidemiologie, München erstellt. Alle anderen Untersuchungen werden in Laboratorien des Klinikums der Universität München durchgeführt. Die Blutproben werden hierzu nur mit dem Verschlüsselungscode weitergegeben und lassen keinen direkten Rückschluss auf den Studienteilnehmer zu. Für die genetischen und epigenetischen Analysen wird eine erneute 2. Verschlüsselung durch die Mitarbeiter des Helmholtz-Zentrums durchgeführt. Diese doppelte Kodierung stellt sicher, dass die genetischen und epigenetischen Daten zusätzlich geschützt werden. Eine Entblindung ist nur durch das Studienzentrum, nicht aber durch die Mitarbeiter des Helmholtz-Zentrums möglich.

Im Falle des Widerrufs der Einwilligung werden der Name und Ihre persönlichen Kontaktdaten aus unserer Datenbank gelöscht. Die bis dahin gespeicherten Daten Ihres Kindes werden nun anonymisiert verwendet. Außerdem werden die Kontaktdaten aller Studienteilnehmer innerhalb eines Monats nach Abschluss der Studie gelöscht. Die schriftlichen Unterlagen, inklusive dieser Einverständniserklärung, werden im Dr. von Haunerschen Kinderspital bis zum Ende der Studie und in einem dafür geeigneten Lager bis zum Ablauf der gesetzlichen Aufbewahrungsfrist (12 Jahre nach Studienende) aufbewahrt.

Im Falle von Veröffentlichungen der Studienergebnisse bleibt die Vertraulichkeit der persönlichen Daten Ihres Kindes ebenfalls gewährleistet, denn die Daten werden, wenn überhaupt, in anonymisierter Form wiedergegeben.

Auf Wunsch werden wir Sie über allgemeine Studienergebnisse informieren.

Im Falle von zusätzlichen, bisher nicht geplanten Untersuchungen oder Datenerhebungen, die über den oben genannten Studienablauf hinausgehen, werden wir das zustimmende Votum der zuständigen Ethikkommission einholen.

Vor der Einwilligung in die Studie haben Sie hier die Möglichkeit gezielt Fragen zu notieren, die noch ausführlicher mit Ihnen besprochen werden sollen.

Einverständniserklärung & Datenschutzerklärung für die Teilnahme meines/unseres Kindes

Einfluss von Milcheiweiß auf das Wachstum und die spätere Entwicklung von Übergewicht (ToMI-Studie)

Name, Vorname des Kindes	Geburtsdatum

Ich erkläre, dass mir die Studienbedingungen vollständig erläutert wurden und alle Fragen zu meiner Zufriedenheit geklärt wurden. Das Formblatt mit den Studieninformationen habe ich erhalten. Ich hatte ausreichend Zeit, dieses Formblatt zu lesen und Fragen zu stellen. Mögliche Risiken und Nachteile für mein Kind wurden mir erklärt. Ich weiß, dass ich jetzt und in Zukunft jede Frage bezüglich dieser Studie und der Untersuchungen stellen kann.
Ich weiß, dass ich/mein Kind jederzeit von der Teilnahme an der Studie zurücktreten kann, ohne dass ich dafür Gründe angeben muss oder dass mir oder meinem Kind Nachteile entstehen würden.

Hiermit willige ich in die Teilnahme meines Kindes in die Studie ein:

Ort, Datum	Name, Vorname 1. Erziehungsberechtigte/r	Unterschrift 1. Erziehungsberechtigte/r

Ich besitze das alleinige Sorgerecht: ☐ Ja ☐ Nein

Ort, Datum	Name, Vorname 2. Erziehungsberechtigte/r	Unterschrift 2. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname Studienpersonal (Aufklärende/r)	Unterschrift Studienpersonal (Aufklärende/r)

Die Datenschutz-Information im Rahmen der Teilnehmerinformation habe ich zur Kenntnis genommen. Ich willige hiermit in die Erhebung und Verwendung der persönlichen Daten meines Kindes nach diesen Maßgaben ein.

Ort, Datum	Name, Vorname 1. Erziehungsberechtigte/r	Unterschrift 1. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname 2. Erziehungsberechtigte/r	Unterschrift 2. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname Studienpersonal (Aufklärende/r)	Unterschrift Studienpersonal (Aufklärende/r)

Einverständnis- & Datenschutzerklärung <u>für die genomweite Genotypisierung und epigenetische Untersuchungen</u> meines/unseres Kindes im Rahmen der ToMI-Studie

Einfluss von Milcheiweiß auf das Wachstum und die spätere Entwicklung von Übergewicht (ToMI-Studie)

Name, Vorname des Kindes	Geburtsdatum

Hiermit willige ich insbesondere ein, dass aus dem Blut meines Kindes **Erbmaterial gewonnen, aufbewahrt und untersucht** werden darf. Die genomweite Genotypisierung, sowie die epigenetischen Untersuchungen dienen der Aufdeckung genetischer Ursachen von Erkrankungen und Ursachen für Übergewicht und Stoffwechselveränderungen im Rahmen der ToMI-Studie. Die Teilnahme an der Untersuchung birgt keine weiteren gesundheitlichen Risiken über die erfolgende Blutentnahme hinaus.

Die Daten und Untersuchungsergebnisse werden ausschließlich für das Untersuchungsziel dieser Studie verwendet. Auf die verschlüsselten Daten können nur autorisierte Mitarbeiter der Studie zugreifen. Eine Weitergabe von Daten an unberechtigte Dritte erfolgt nicht. Die im Rahmen dieser Studie gewonnenen genetischen Daten werden bis zu 10 Jahren nach Abschluss der wissenschaftlichen Untersuchung oder bis auf Widerruf aufbewahrt.

Ich weiß, dass ich jetzt und in Zukunft weitere Fragen bezüglich dieser Studie und den einzelnen Untersuchungen stellen kann. Ich weiß, dass ich jederzeit von der freiwilligen Teilnahme an der Studie zurücktreten kann, ohne dass ich hierfür Gründe angeben muss.

Ich willige freiwillig in die Erhebung, Verarbeitung und Nutzung personenbezogener Daten nach Maßgabe des Aufklärungsbogens der Studie ein. Für die Erhebung, Verarbeitung und Nutzung ist der Leiter des Forschungsvorhabens, Herr Prof. Berthold Koletzko, verantwortlich.

Ort, Datum	Name, Vorname 1. Erziehungsberechtigte/r	Unterschrift 1. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname 2. Erziehungsberechtigte/r	Unterschrift 2. Erziehungsberechtigte/r
Ort, Datum	Name, Vorname Studienpersonal (Aufklärende/r)	Unterschrift Studienpersonal (Aufklärende/r)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Check/page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Y
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	11
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 13
	5b	Name and contact information for the trial sponsor	13
	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	13
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13
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
	6b	Explanation for choice of comparators	
Objectives	7	Specific objectives or hypotheses	4
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, noninferiority, exploratory)	4

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
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)	4, Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4, Table 1
	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)	5
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
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 relevance of chosen efficacy and harm outcomes is strongly recommended	5,6
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)	7, 12, Figure 1
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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	7

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	7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
Methods: Data collection, management, and analysis			
Data collection methods	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 validity, if known. Reference to where data collection forms can be found, if not in the protocol	7,8
	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	5
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	8,9,11
Statistical methods	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	9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
	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)	9
Methods: Monitoring			
Data monitoring	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	10

	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	10
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	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	10
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
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)	11
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	12
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	8,12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
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	11
Dissemination policy	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	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	12
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	12

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplement
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	6

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.