PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Grote, Veit; Jaeger, Vanessa; Escribano, Joaquin; Zaragoza, Marta; Gispert, Mariona; Grathwohl, Dominik; Koletzko, Berthold

VERSION 1 – REVIEW

REVIEWER	Anderson, Christopher Public Health Foundation Enterprises Women Infants and Children
REVIEW RETURNED	02-Feb-2021

GENERAL COMMENTS	This will be a very interesting study with results that could have great impact on efforts to control childhood obesity. The protocol is detailed and well written, and I have a few very minor suggestions for clarification. 1. Including that the primary outcome of the study is growth between 12 and 24 months of age in the title might be of benefit. 2. The main objective for the study suggests that the effect of toddler milk protein content on growth during the second year of life is the outcome- this suggests to me that the outcome will be the change in BMIz between baseline and 24 months of age. If this is the case, the outcome should be described elsewhere as the change in BMIz from baseline. This also applies to the other outcomes: are they evaluated as final values, or changes from baseline values (for those that were measured at baseline)? 3. Sample size: When describing the effect size (not the absolute value) of BMIz, including the units (SD) with the numeric value would provide clarity (0.2 SD instead of 0.2). 4. Sample size: "higher or lower formula" should be revised to "higher or lower protein content formula" 5. Sample size (2nd paragraph): "The sample size was calculated with the BMI" needs to indicate that this is the anticipated effect size (0.15 SD) 6. Sample size: It appears that the power analysis for the secondary endpoint (72 months) assumes no reduction in effect size over the additional 48 elapsed months. Perhaps replace "also a difference" with "a difference of the same magnitude" 7. Allocation: I interpreted "balanced randomization" to mean blocked randomization. If this is the case, some additional information (block size) may be helpful. 8. Data collection and management: "dietary intake records" obfuscates that the food frequencies are collected as part of the study- data may be more clear than records

	9. Statistical methods: the full analysis dataset as described deviates from an intention to treat analysis. It may be important to justify why ITT was not considered. 10. Data monitoring: the non-inferiority for growth section is a bit difficult to understand as currently written, and may benefit from inclusion of the used non-inferiority margin. 11. The informed consent documents do not appear to be attached as a supplement (as indicated in the SPIRIT checklist)

REVIEWER	Merritt, Russell J University of Southern California
REVIEW RETURNED	16-Jun-2021

GENERAL COMMENTS	The research addresses what may be an important question. There is considerable data on the importance of cow milk protein for the growth of some populations and concerns regarding adverse metabolic effects. The protocol is generally clearly written, and the study methods support the objectives. A few questions remain: 1. The stated objective is to determine the long term impact of differences in protein intake in the second year of life. The subjects are followed to 6 years. It is not clear why the primary objective is BMI at 2 years, rather than 6 years. 2. The subjects will have had variable exposure to human milk and infant formulas in the first year of life. There is a lack of detail as to how these historical differences will be incorporated into the analysis of the effect of the second year of life intervention. 3. The "Control" formula is higher in protein per 100 Cal than cow milk. Is there a rationale for this level of protein in the formula? 4. Please clarify the composition of the cow milk protein. Is it modified in terms of whey and casein content from standard cow milk? 5. How will subject compliance with the guideline of a minimum of 300 ml formula per day influence how the data are to be analyzed. 6. The dietary assessment plan lacks detail. Will food frequency questionnaires and 24-hour recall be done at each dietary assessment time point? It would be helpful to provide some justification of the dietary assessment methods selected for the age groups studied. 7. Please discuss any risk of bias and the implications for generalizabiilty in the processes for subject recruitment (as to their past nutrition history). 8. Please state that the two formulas are prepared the same way by the subjects' families. 9. The protocol indicates that a powdered formula is used. The table of formula composition indicates "ready to feed," not "as prepared." 10. The method to be used to assess DNA methylation is not described. 11. In the section of statistical methods, the term "Stage 3" seems unclear as to what it signifies.

REVIEWER	Uthaya, Sabita Imperial College London
REVIEW RETURNED	19-Jun-2021

GENERAL COMMENTS	I would have liked to have seen details about the formulae to be used in this study. By whom and where were these manufactured and any safety data / previous use. Are they manufactured by a

	commercial company and if so what the role of the company is? It was also unclear who the Sponsor of the study is.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Christopher Anderson, Public Health Foundation Enterprises Women Infants and Children
Comments to the Author:
This will be a very interesting study with results that could have great impact on efforts to control childhood obesity. The protocol is detailed and well written, and I have a few very minor suggestions for clarification.

1. Including that the primary outcome of the study is growth between 12 and 24 months of age in the title might be of benefit.
We changed the title accordingly. “Effect of milk protein content in toddler formula on later BMI and obesity risk: Protocol of a multicentre randomized controlled trial (ToMI)”

2. The main objective for the study suggests that the effect of toddler milk protein content on growth during the second year of life is the outcome- this suggests to me that the outcome will be the change in BMIz between baseline and 24 months of age. If this is the case, the outcome should be described elsewhere as the change in BMIz from baseline. This also applies to the other outcomes: are they evaluated as final values, or changes from baseline values (for those that were measured at baseline)?
The outcome is as described but indeed adjusted by baseline value – so also described in the method section. The outcome is explicitly not change in BMI (BMI_{24mo}-BMI_{12mo}). Nevertheless, this is only a methodological question as the result is almost identical but variation in BMI is tackled differently. Generally, the adjustment method is considered a better way to analyse change. We added the adjustment for baseline in the abstract and outcome section. Furthermore, we also added BMI to the introduction of the abstract.

3. Sample size: When describing the effect size (not the absolute value) of BMIz, including the units (SD) with the numeric value would provide clarity (0.2 SD instead of 0.2).
Was added.
4. Sample size: "higher or lower formula" should be revised to "higher or lower protein content formula"
Was added.

5. Sample size (2nd paragraph): "The sample size was calculated with the BMI" needs to indicate that this is the anticipated effect size (0.15 SD)
We changed the sentence accordingly.

6. Sample size: It appears that the power analysis for the secondary endpoint (72 months) assumes no reduction in effect size over the additional 48 elapsed months. Perhaps replace "also a difference" with "a difference of the same magnitude"
We changed the sentence accordingly.

7. Allocation: I interpreted "balanced randomization" to mean blocked randomization. If this is the case, some additional information (block size) may be helpful.
No, “balanced allocation” is different to block randomization. As only country was included as a strata the balanced randomization randomly assigns the product codes in each country to randomized

subjects but guarantees that no code is randomly assigned by chance too often. It is a standard randomization method.

8. Data collection and management: "dietary intake records" obfuscates that the food frequencies are collected as part of the study- data may be more clear than records
We clarified the nutritional assessment, as it was not clear that 24-recalls and FFQs were collected at different time points.

9. Statistical methods: the full analysis dataset as described deviates from an intention to treat analysis. It may be important to justify why ITT was not considered.
We added a sentence that FAS is as close as possible/reasonable to ITT.

10. Data monitoring: the non-inferiority for growth section is a bit difficult to understand as currently written, and may benefit from inclusion of the used non-inferiority margin.
We tried to re-phrase the section and added the margin.

11. The informed consent documents do not appear to be attached as a supplement (as indicated in the SPIRIT checklist)
Sorry, we forgot to upload the ICF. Now it is added and referred to in the main manuscript.

Reviewer: 2
Dr. Russell J Merritt, University of Southern California Comments to the Author:
The research addresses what may be an important question. There is considerable data on the importance of cow milk protein for the growth of some populations and concerns regarding adverse metabolic effects. The protocol is generally clearly written, and the study methods support the objectives. A few questions remain:
1. The stated objective is to determine the long term impact of differences in protein intake in the second year of life. The subjects are followed to 6 years. It is not clear why the primary objective is BMI at 2 years, rather than 6 years.
Traditionally outcomes are measured directly after an intervention period. We were happy to have a sponsor that supports long-term follow-up until 6 years of age which is not typical in this setting. It gives the opportunity to see if any effects are also seen at later ages. However, long term follow-up has a high risk of loss to follow-up due to the fact that we follow a healthy population in a setting without major benefits for the participants.

2. The subjects will have had variable exposure to human milk and infant formulas in the first year of life. There is a lack of detail as to how these historical differences will be incorporated into the analysis of the effect of the second year of life intervention.
The historical differences are currently not planned to be incorporated in the primary analysis. We did and do not expect that the “historical data” interact with the intervention. Nevertheless, these data will be analysed and will be related to relevant outcomes to understand their short and long term effects on growth and metabolism. We added therefore a short paragraph on secondary objectives of the trial that alludes to the fact that we will use the study data also in the sense of a cohort study to address relevant questions.

3. The "Control" formula is higher in protein per 100 Cal than cow milk. Is there a rationale for this level of protein in the formula?
The level of protein in our control toddler milk (6.15 g protein / 100 kcal) is somewhat lower than that of 2% fat cow’s milk which is the type of milk the study product and typical toddler milk on the market is aiming at. According to the UDSA Food Composition Database, reduced fat cow’s milk (2% milk fat) contains about 6.72 g protein / 100 kcal. We now added “2%” standard cow’s milk to make this clear. Furthermore, we added that the formulas are based on cow’s milk and have the

same casein:whey protein ratio.
4. Please clarify the composition of the cow milk protein. Is it modified in terms of whey and casein content from standard cow milk?
As the product is purely based on cow's milk protein there was no adaptation of the whey:casein ration.

5. How will subject compliance with the guideline of a minimum of 300 ml formula per day influence how the data are to be analyzed.
We better defined the per-protocol group in the manuscript to clarify this.

6. The dietary assessment plan lacks detail. Will food frequency questionnaires and 24-hour recall be done at each dietary assessment time point? It would be helpful to provide some justification of the dietary assessment methods selected for the age groups studied.
We added a respective paragraph that explains the rationale and clarified the timing.

7. Please discuss any risk of bias and the implications for generalizabilty in the processes for subject recruitment (as to their past nutrition history).
We are not fully sure what the question is aiming at. For sure, the selected population is not a general population, as participants in such a trial will be selected and have specific interest in nutrition and research. As most children have some kind of milk (formula or cow's) at the time of randomization, we believe that this a less problematic point for selection bias. If any historical feeding is influencing the effect of the intervention is – to our knowledge – not known.
We have difficulties to find a section in the manuscript to include such potential biases.

8. Please state that the two formulas are prepared the same way by the subjects' families.
We added that all product codes received the same instructions for preparation.

9. The protocol indicates that a powdered formula is used. The table of formula composition indicates "ready to feed," not "as prepared."
Thank you, yes that is correct since "ready to feed" is reserved for manufacturer prepared milk, we changed to "as prepared".

10. The method to be used to assess DNA methylation is not described.
Currently, we still do not have funding for DNA methylation. It is only planned as an option provided additional funding can be secured, and hence methodology would have to be defined at the time when decisions are made on this option. For example, in recent years the most commonly used methodology changed markedly from the Illumina 450k to the Illumina850k approach. Therefore, no detailed method is described at this time.
We added a sentence to the text to clarify this aspect?

11. In the section of statistical methods, the term "Stage 3" seems unclear as to what it signifies.
Indeed unclear. In the protocol Stage 3 was defined as the analysis at 6 years of age. So we changed to "6 years of age"

Reviewer: 3
Miss Sabita Uthaya, Imperial College London Comments to the Author:
I would have liked to have seen details about the formulae to be used in this study. By whom and where were these manufactured and any safety data / previous use. Are they manufactured by a commercial company and if so what the role of the company is? It was also unclear who the Sponsor of the study is.
The sponsor is named within the funding section of the manuscript. The sponsor is Nestle, formerly named Nestec. The sponsor is also the producer of the milk which was described in the "role of the

sponsor" section. We now added some additional information in the formula section. The milk was not used in any study beforehand and was not in commercial use before study start.

Reviewer: 1
Competing interests of Reviewer: None declared

Reviewer: 2
Competing interests of Reviewer: I have worked for infant formulas companies in the past, including the sponsor of this study.

Reviewer: 3
Competing interests of Reviewer: None

VERSION 2 – REVIEW

REVIEWER	Anderson, Christopher Public Health Foundation Enterprises Women Infants and Children
REVIEW RETURNED	19-Sep-2021

GENERAL COMMENTS	The authors have sufficiently addressed my comments in this revision of the protocol. I offer the following minor suggestion that may provide additional clarity for readers. Minor comments: 1. Page 10, line 15: "a two-way interaction between child age and intervention group" might be more clear in the place of "age times intervention group"

REVIEWER	Merritt, Russell J University of Southern California
REVIEW RETURNED	30-Sep-2021

GENERAL COMMENTS	The reviewer appreciates the clarifications provided by the investigators and the noted changes to the protocol manuscript. My remaining comments are as follows: It might be helpful to explain the abbreviation TOMI in the title. It is currently unclear from the title how that was derived, although it is explained later. This reviewer expressed concerns regarding any potential influence of feeding mode in the first year of life on the planned intervention in the second year of life. The response of the authors is not fully consistent with the first sentence of the Introduction of the manuscript, but hopefully, adjusted analyses will prove feasible to examine any interactions. The secondary study objectives remain vague, but there appears to be ongoing development of the data analysis plan. Some of the metabolic markers examined may be affected by recent intake. Is there a plan to control the timing of blood draws as related to the time of the last meal?

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Dr. Christopher Anderson, Public Health Foundation Enterprises Women Infants and Children
Comments to the Author:
The authors have sufficiently addressed my comments in this revision of the protocol. I offer the following minor suggestion that may provide additional clarity for readers.
Minor comments:
1. Page 10, line 15: “a two-way interaction between child age and intervention group” might be more clear in the place of “age times intervention group”
We changed the sentence accordingly.

Reviewer: 2
Dr. Russell J Merritt, University of Southern California
Comments to the Author:
The reviewer appreciates the clarifications provided by the investigators and the noted changes to the protocol manuscript. My remaining comments are as follows:
It might be helpful to explain the abbreviation TOMI in the title. It is currently unclear from the title how that was derived, although it is explained late.

We changed the title to: “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”

This reviewer expressed concerns regarding any potential influence of feeding mode in the first year of life on the planned intervention in the second year of life. The response of the authors is not fully consistent with the first sentence of the Introduction of the manuscript, but hopefully, adjusted analyses will prove feasible to examine any interactions.

Re-reading the first sentence of the introduction, we still do not understand fully what point the reviewer is making. However, the cited intervention study never showed that the effect of the first-year intervention is increasing in the second year of life but is sustained until 2 years of life. Weight gain up to 2 years is - according the literature -one of the strongest risk factors for later BMI and was the primary outcome of the cited study. However, the lower weight gain in the first 2 years of life was archived in the early phase of the intervention in the cited study, not in the second year of life. Nevertheless, we certainly will consider baseline differences or mayor growth modulating factors in our sensitivity analysis, for adjustment and effect modifications.

The secondary study objectives remain vague, but there appears to be ongoing development of the data analysis plan.
Some of the metabolic markers examined may be affected by recent intake. Is there a plan to control the timing of blood draws as related to the time of the last meal?

The time since the last meal was collected to make potential adjustments and sensitivity analysis. However, currently the statistical analysis plan is not mentioning the adjustment, while it is clearly planned and therefore collected! Thanks for pointing this out. As we have not included it so far in any official trial document, I am reluctant to include it in this manuscript.

Reviewer: 1
Competing interests of Reviewer: None.

Reviewer: 2
Competing interests of Reviewer: The reviewer has previously worked for companies manufacturing infant formulas, including the sponsor of this study.