PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A 16-week multicenter randomized controlled trial to study the effect of the consumption of an oat beta-glucan enriched bread versus a wholegrain wheat bread on glycemic control among persons with pre-diabetes- a study protocol of The CarbHealth study
AUTHORS	Hjorth, Therese; Schadow, Alena; Revheim, Ingrid; Spielau, Ulrike; Thomassen, Lise M.; Meyer, Klara; Piotrowski, Katja; Rosendahl-Riise, Hanne; Rieder, Anne; Varela, Paula; Lysne, Vegard; Ballance, Simon; Koerner, Antje; Landberg, Rikard; Buyken, Anette; Dierkes, Jutta

VERSION 1 – REVIEW

REVIEWER	Fogacci, Federica University of Bologna
REVIEW RETURNED	27-Mar-2022

GENERAL COMMENTS	I carefully read the manuscript by Hjorth et al. My comments and suggestions for the authors are the following: - Page 4, Lines 7-9: "as it would be a difficult undertaking for one study center alone". I suggest the authors to remove this sentence. As a matter of fact, the inclusion criteria are not particularly stringent. Definitely, these are patients who shouldn't be very hard to find. - In their protocol, the authors should more comprehensively refer to availble literature. For example, they should cite doi: 10.3390/nu12030686. - Liver transaminases should be better reported as AST and ALT instead of ALAT and ASAT. - Statistical analysis should be better described. The authors should refer to the specific statistical tests they planned to perform.

REVIEWER	Westerterp-Plantenga, Margriet University of Maastricht
REVIEW RETURNED	16-Apr-2022

GENERAL COMMENTS	The manuscript titled: A 16-week multicenter randomized controlled trial to study the effect of the consumption of an oat beta-glucan enriched bread versus a wholegrain wheat bread on glycemic control among persons with pre-diabetes – The CarbHealth study described the protocol of the study very clearly. The study named The CarbHealth trial is a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants 40-70 years of age with overweight and prediabetes. The study is being conducted at four universities in Norway, Sweden and Germany, with 250 participants. The primary

	outcome is the difference in HbA1c between the intervention and the control group. The study has been registered as a clinical trial, and ethics have been taken into account adequately. The study implies several strengths, and some weaknesses. Strengths are the following aspects: - a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants 40-70 years of age with overweight and prediabetes. - the feasibility of the study - keeping the participants at stable body weight. - the 16 weeks duration, with a measurement timepoint at 8 weeks; 16 weeks is sufficient to measure a change in body composition. - the indicated measurements of physical activity, chronotype, fasting blood glucose and serum lipid profile, body weight, hepatic steatosis markers, 24 h glucose profiles, gastric emptying, changes in microbiota, consumer acceptance and attrition rates. Weaknesses are the following: - body composition measurements with BIA - lack of a biomarker for dietary compliance - lack of a biomarker of energy intake - limited accelerometer measurements of physical activity - use of an unvalidated PA questionnaire Clarifications - Is the accelerometer used for chronotype an Actigraph Sleep type? Would it be possible to use it in all participants? Suggestions: Knowing that the study protocol was granted and that the study has started, perhaps some additions to measurements or data analyses still might be helpful. - Weight stability is of utmost importance, since weight loss is likely to affect HbA1c concentrations. Could it be monitored more continuously? - If possible, body composition might be measured using Bodpod, or hydrodensitometry, or deuterium dilution, or DEXA at more centers, since the deviations obtained by BIA measurements in participants with overweight are well-documented. - Would it be possible to use e.g. metabolomics as biomarker for dietary compliance? - Energy intake can be estimated using an equation for BMR based upon body composition, multiplied by Physical Activity Level derived from accelerometer counts. See Drummen et al. AJCN 114; 1847-1858; 2021. It then is advised to only use the dietary recalls that are within a range of energy intake of +/- 10% of estimated energy intake. - Perhaps, in addition to the PA questionnaire that is used, the Baecke questionnaire could be implied, since it is the only DLW validated PA questionnaire. - With respect to the importance of attrition rate and its effects, the following might be helpful: Tanja C. Adam, et al.,Diabetes Care 2021;44(7):1491–1498 - With respect to associations with HbA1c, it may be helpful to know about previous associations of HbA1c and dietary protein. Drummen et al. AJCN 114; 1847-1858; 2021.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Federica Fogacci, University of Bologna Comments to the Author:
Dear Editor,
I carefully read the manuscript by Hjorth et al.
My comments and suggestions for the authors are the following:
Thank you for your comments and suggested revision! Please, see below our answers to your comments and suggested revisions.

- Page 4, Lines 7-9: "as it would be a difficult undertaking for one study center alone". I suggest the authors to remove this sentence. As a matter of fact, the inclusion criteria are not particularly stringent. Definitely, these are patients who shouldn't be very hard to find.
Thank you. We agree and we have now removed this sentence on page 4 as well as on page 16.

- In their protocol, the authors should more comprehensively refer to availble literature. For example, they should cite doi: 10.3390/nu12030686.
Thank you for your suggestion. We have now included the suggested literature (pls see page 5 in the updated manuscript.)

- Liver transaminases should be better reported as AST and ALT instead of ALAT and ASAT.
Thank you, this has been adjusted on page 15 in the updated manuscript.
- Statistical analysis should be better described. The authors should refer to the specific statistical tests they planned to perform.

Thank you! We have now further clarified the planned statistical analysis (pls see page 15-16 in the updated manuscript).

Reviewer: 2
Prof. Margriet Westerterp-Plantenga, University of Maastricht Comments to the Author:
The manuscript titled: A 16-week multicenter randomized controlled trial to study the effect of the consumption of an oat beta-glucan enriched bread versus a wholegrain wheat bread on glycemic control among persons with pre-diabetes – The CarbHealth study described the protocol of the study very clearly. The study named The CarbHealth trial is a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants 40-70 years of age with overweight and prediabetes. The study is being conducted at four universities in Norway, Sweden and Germany, with 250 participants. The primary outcome is the difference in HbA1c between the intervention and the control group. The study has been registered as a clinical trial, and ethics have been taken into account adequately.
The study implies several strengths, and some weaknesses.
Strengths are the following aspects:
- a multi-center double-blind randomized controlled 16-week dietary intervention trial in participants 40-70 years of age with overweight and prediabetes.
- the feasibility of the study
- keeping the participants at stable body weight.
- the 16 weeks duration, with a measurement timepoint at 8 weeks; 16 weeks is sufficient to measure a change in body composition.
- the indicated measurements of physical activity, chronotype, fasting blood glucose and serum lipid profile, body weight, hepatic steatosis markers, 24 h glucose profiles, gastric emptying, changes in microbiota, consumer acceptance and attrition rates.
Weaknesses are the following:
- body composition measurements with BIA
- lack of a biomarker for dietary compliance
- lack of a biomarker of energy intake
- limited accelerometer measurements of physical activity

- use of an unvalidated PA questionnaire Clarifications
- Is the accelerometer used for chronotype an Actigraph Sleep type? Would it be possible to use it in all participants?
We would like to thank the reviewer for excellent comments and suggestions. Unfortunately, many of the suggested changes are difficult to implement since the study is well head already and will be finalized before Summer 2023. However, we incorporated as many of the suggestions as possible in the current/ongoing trial. Pls see below our replies to the reviewer's comments and suggestions.

In Paderborn we use Empatica accelerometer which the participants wear during the same week as they wear the CGM. In the other centers we only use MCTQ

Suggestions:
Knowing that the study protocol was granted and that the study has started, perhaps some additions to measurements or data analyses still might be helpful.
- Weight stability is of utmost importance, since weight loss is likely to affect HbA1c concentrations. Could it be monitored more continuously?
We do monitor weight stability by measuring body weight at study week 0, 8 and 16, and can account for changes that may/will occur as one can expected due to its nature of effectiveness trial. Furthermore, since participants were asked to replace breads we were not expecting any major changes in bodyweight during the trial. Moreover, the study is an effectiveness ("pragmatic study") in which we are evaluating the impact of a bread replacement in the habitual diet on primary and secondary endpoints.

- If possible, body composition might be measured using Bodpod, or hydrodensitometry, or deuterium dilution, or DEXA at more centers, since the deviations obtained by BIA measurements in participants with overweight are well-documented.
Since body composition is not a primary outcome, we decided that it was not necessary to use the same method in the centers. However, In Bergen we are using a Bodpod and comparative evaluations is possible for approximately 100 persons, in Gothenburg we are using a DEXA and comparative evaluations is possible for approximately 50 persons.

- Would it be possible to use e.g. metabolomics as biomarker for dietary compliance?
We appreciate this comment, and we are very interested in using metabolomics both to assess potential differences due to treatment that could give us mechanistic insights, differentiate responders and non-responders to intervention as well as to reflect dietary intakes and potentially also compliance. We have included explorative analysis using plasma metabolomics (untargeted). With this approach we will be able to find potential biomarkers of oats intake. Moreover, we are conducting separate controlled feeding trials to discover new oat biomarkers and to evaluate putative oat biomarkers (avenanthramides and avenacosides) and the samples from the current study will be used to validate the findings (but that will happen first 2024-2025).

- Energy intake can be estimated using an equation for BMR based upon body composition, multiplied by Physical Activity Level derived from accelerometer counts. See Drummen et al. AJCN 114; 1847-1858; 2021. It then is advised to only use the dietary recalls that are within a range of energy intake of +/- 10% of estimated energy intake.
Again, this is an effectiveness trial (pragmatic) and bodyweight and body composition are not a primary outcome, therefore we have chosen not to add more measures at this stage of the trial.

- Perhaps, in addition to the PA questionnaire that is used, the Baecke questionnaire could be implied, since it is the only DLW validated PA questionnaire.
Thank you for the recommendation of Baecke questionnaire. We are using the IPAQ questionnaire to assess physical and sedentary behavior, since IPAQ was considered to have acceptable measurement properties. We are aware of the benefits of the Baecke questionnaires, and we will discuss the potential limitations of using IPAQ by the end of the trial in upcoming publications.

- With respect to the importance of attrition rate and its effects, the following might be helpful: Tanja C. Adam, et al.,Diabetes Care 2021;44(7):1491–1498 Thank you for your suggestion. The attrition rate for the CarbHealth study varies slightly between sites and is currently approximately 15-20%, whereas the PREVIEW study had approximately 25%. In the power calculation we used data from other dietary intervention trials and estimated the drop out to be 45%, but that will not be the case. We will carefully analyze the effects of attrition rates. Many thanks for the suggested reading!

- With respect to associations with HbA1c, it may be helpful to know about previous associations of HbA1c and dietary protein. Drummen et al. AJCN 114; 1847-1858; 2021.
Thank you for the suggestion. We will keep this in mind for future publications.

VERSION 2 – REVIEW

REVIEWER	Fogacci, Federica University of Bologna
REVIEW RETURNED	21-Jul-2022

GENERAL COMMENTS	I carefully read the revised version of the paper and authors' reply to my concerns. I have no further comments.

REVIEWER	Westerterp-Plantenga, Margriet University of Maastricht
REVIEW RETURNED	06-Jul-2022

GENERAL COMMENTS	Comments have been addressed adequately.