PEER REVIEW HISTORY

BMJ Medicine publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to a another journal from BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Medicine. The paper was subsequently accepted for publication at BMJ Medicine.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between Coeliac disease and Cardiovascular Disease: A prospective analysis in UK Biobank
AUTHORS	Conroy, Megan; Allen, Naomi; Lacey, Ben; Soilleux, Elizabeth; Littlejohns, Thomas

VERSION 1 - REVIEW

REVIEWER 1	Holmes, Geoffrey K T; Royal Derby Hospital. Competing Interest: None
REVIEW RETURNED	12-Aug-2022

GENERAL COMMENTS	As the authors point out research on the risk of those with coeliac disease (CD) developing cardiovascular disease (CVD) is extensive but conclusions drawn are often conflicting. They therefore, designed this prospective study to re-assess this risk for CVD that included ischaemic heart disease (IHD), myocardial infarction (MI) and stroke. Information was gleaned from the UK Biobank Cohort. This enabled large numbers of individuals to be considered, 467,012 subjects without CD and 2,083 with CD were included in the analysis. Serum biomarkers relevant to CVD and risk factors were considered in the assessment. Incident rates for CVD were calculated and stratified by risk score group generated from a modified version of the American Heart Association's Life's Simple Seven Score (LS7); ideal, intermediate and poor. Exploration of a possible association between risk of CVD and length of diagnosis was undertaken. CD was associated with a 27% increased risk of CVD. Risks were elevated for IHD and MI but not for stroke. Those with CD were more likely to have an ideal CVD risk score compared to those without CD (23.3% v 14.3%). Systemic inflammation as measured by C-reactive protein (CRP) did not appear to explain the findings. There was a slight reduced risk for those who had CD for <10years (30%) compared to those with CD >10 years (34%). I have some comments: 1. This is a well conceived study. The paper is well written with informative tables. 2. Interesting data are presented particularly that traditional risk factors for CVD are low in CD but CVD is elevated in this group. Systemic inflammation might still be important here but CRP not sensitive enough to detect it as the authors recognise. 2. It seems that if a prevalence of 1% is accepted for CD in the community then the number of those with CD (2083) in this large group is rather low .Could the authors comment.

	3. Since diabetes is associated with CD it is surprising that the percentage (3.2%) it so much lower than in the no-CD group (4.4%). Again could the authors comment. 4. The first paragraph of the Introduction could be omitted since it does not relate to the study and would shorten the paper a little. The Introduction could start with the second paragraph. 5. The final paragraph of the Discussion advises that clinicians should monitor CD patients for CVD. How could this be done in practice? The authors believe that this is the first such study to employ a CVD risk score and I think that they are correct in this assumption. They were also able to assess risk against length of CD diagnosis which I think is novel. Health Care workers who look after those with CD need to be aware that CVD is elevated in CD so that optimum treatment can be given and especially preventive measures instituted. Since many CD patients are now looked after by GPs, nurses or dieticians rather than in gastroenterology clinics, publication of this work in a general journal would seem appropriate. I believe the information presented is reliable and of interest to all working in the CD field. It should spur further research in this interesting aspect of CD.

REVIEWER 2	Ciaccio, Edward J; Columbia University. Competing Interest: None
REVIEW RETURNED	12-Aug-2022

GENERAL COMMENTS	I am not happy with this. It is based in part on questionnaire. CD is self-reported and they only ask about wheat: Further, a gluten free diet was not taken into account as the diet questionnaire only asked if a wheat free diet was followed (gluten is identified in other grains, such as barley and rye) and the numbers of participants with CD who reported not following a wheat free diet was small, so the impact of a gluten free diet could not be assessed. Consider this paper (I am not co-author) Body mass index in celiac disease: beneficial effect of a gluten-free diet Jianfeng Cheng 1, Pardeep S Brar, Anne R Lee, Peter H R Green Doesn't seem to have been discussed or cited. The authors also missed key reviews on this topic. CD can have difficulties like nutritional deficiency and overweight.

REVIEWER 3	De Marchi, Sergio; Dept of Medicine - University of Verona. Competing Interest: None
REVIEW RETURNED	12-Aug-2022

GENERAL COMMENTS	The paper adresses a very interesting and challenging issue regardin coeliac disease (CD) and more in general the interaction between immune disorders and cardiovascula disease. The study is well drawn and the paper comes to fair conclusions . The numerosity of the population is robust and the analysis

	accurate. CD patients have an increased risk of CVD and in particular of cardiovascular events , interestingly no correlation with cerebrovascular disease, infact this pathology is mainly connected with hypertension and in CD patients the prevalence is actually low . Some minor issues I think authors should address (or stress) in discussion : 1. despite no traditional CVD risk factors seem linked with CD , neither traditional infammatory marker such as PCR, I think it should be stressed in discussion that adhesion to diet prescription is necessary to lower inflammation and the study can not give data about this point , may be other markers (IL-6) may be more efficient in this case 2. low adherence to diet (declared 84%) may determine impaired absorption of vitamins (D or E or other) that may influence CVD risk . 3. wheat free diet may have increased level of saturated fat or fat and salt and this aspect should be considered indiscussion

REVIEWER 4	Kaukinen, Katri; Tampere University. Competing Interest: Academic research funding from the Academy of Finland, Finnish private Foundations.
REVIEW RETURNED	12-Aug-2022

GENERAL COMMENTS	Strengths of the study: prospective design, large study cohort, results have been adjusted with cardiovascular risk factors, application of cardiovascular risk score. However, previous knowledge/studies should be cited better. There is already one previous UK Biobank study on celiac disease showing increased risk for cardiovascular diseases (CVD) and increased risk for cardiovascular mortality in patients with celiac disease (Schneider CV, Kleinjans M, Fromme M, Schneider KM, Strnad P. Phenome-wide association study in adult coeliac disease: role of HLA subtype. Aliment Pharmacol Ther. 2021 Feb;53(4):510-518.). Furthermore, recent study on the association between celiac disease and CVD with data adjusted for common cardiovascular risk factors is missing (Naaraayan A, Nimkar A, Jesmajian S, Gitler B, Acharya P Atherosclerotic Cardiovascular Disease Prevalence Among Patients With Celiac Disease in the United States: An Observational Study. Mayo Clin Proc. 2021 Mar;96(3):666-676.). In addition, previous findings of professor Ludvigsson's group from Sweden on celiac disease and CVD should be acknowledged better. Their recent large JAMA study (ref no 16; JAMA 2020 Apr 7;323(13):1277-1285) with 50.000 celiac disease patients showed that individuals with celiac disease were at increased risk of death from cardiovascular disease. And they have previously shown (ref no 12, Aliment Pharmacol Ther 2013 May;37(9):905-14) that despite evidence of an increased risk of ischemic heart disease and higher cardiovascular mortality, patients with celiac disease with ischemic heart disease have a more favorable cardiac risk profile compared with ischemic heart disease in reference individuals. The paper could be shortened, and text condensed (especially in

	the discussion; first paragraph repeats the findings, it has no references). References: some references are given twice (e.g no 12 and 39 as well as 15 and 35 are the same). Some references could be omitted (e.g not so many general review articles, or studies on the incidence and prevalence of celiac disease are needed here). References no 25 and 26 are inadequate. Table 1: wheat-free diet: Wording “gluten-free diet” would be correct (celiac disease patients are on a gluten-free diet, they are not avoiding only wheat). Table 2 and text in the results:” Celiac disease patients were diagnosed less likely with diabetes”. according to several studies and meta-analyses, patients with celiac disease have increased risk for type 1 diabetes mellitus and patients with type 1 diabetes have increased risk for celiac disease. The situation is different in type 2 diabetes. It would be better to give data separately on type 1 and type 2 diabetes mellitus.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Recommendation:

Comments:
As the authors point out research on the risk of those with coeliac disease (CD) developing cardiovascular disease (CVD) is extensive but conclusions drawn are often conflicting. They therefore, designed this prospective study to re-assess this risk for CVD that included ischaemic heart disease (IHD), myocardial infarction (MI) and stroke. Information was gleaned from the UK Biobank Cohort. This enabled large numbers of individuals to be considered, 467,012 subjects without CD and 2,083 with CD were included in the analysis. Serum biomarkers relevant to CVD and risk factors were considered in the assessment. Incident rates for CVD were calculated and stratified by risk score group generated from a modified version of the American Heart Association's Life's Simple Seven Score (LS7); ideal, intermediate and poor. Exploration of a possible association between risk of CVD and length of diagnosis was undertaken.
CD was associated with a 27% increased risk of CVD. Risks were elevated for IHD and MI but not for stroke. Those with CD were more likely to have an ideal CVD risk score compared to those without CD (23.3% v 14.3%). Systemic inflammation as measured by C-reactive protein (CRP) did not appear to explain the findings. There was a slight reduced risk for those who had CD for <10years (30%) compared to those with CD >10 years (34%).

I have some comments:
1. This is a well conceived study. The paper is well written with informative tables.

No response required
2. Interesting data are presented particularly that traditional risk factors for CVD are low in CD but CVD is elevated in this group. Systemic inflammation might still be important here but CRP not sensitive enough to detect it as the authors recognise.

No response required
2. It seems that if a prevalence of 1% is accepted for CD in the community then the number of those with CD (2083) in this large group is rather low .Could the authors comment.

DONE: Prior to the exclusions being applied, the prevalence of coeliac disease in UKB is 0.65%, which is lower than that accepted community prevalence, and this is likely to be due to UKB being affected by the "health volunteer effect", as mentioned in our limitations section. We have added the following to our manuscript:

Lines 404-405: "affected by the "healthy volunteer effect" [33],which may explain the lower prevalence of CD seen in this study compared to the general population"

3. Since diabetes is associated with CD it is surprising that the percentage (3.2%) it so much lower than in the no-CD group (4.4%). Again could the authors comment.

DONE: Following another reviewer's recommendation, we have subtyped diabetes into type 1, type 2 and unspecified. This is complex using the data UKB have collected (see https://doi.org/10.1371/journal.pone.0162388 for more information), but can be accurate for self-reported diabetes. This has highlighted that there is an increased risk for type 1 diabetes in those that have CD, which is in line with previous research. A decreased risk is seen for type 2 diabetes, and given the overwhelming majority of diabetic participants are type 2, this leads to the overall decrease in risk seen. We have added these results to Table 2 and added the following to the results:

Lines 234 – 235 "less likely to be diagnosed with type 2 diabetes, more likely to be diagnosed with type 1 diabetes"

4. The first paragraph of the Introduction could be omitted since it does not relate to the study and would shorten the paper a little. The Introduction could start with the second paragraph.

DONE

5. The final paragraph of the Discussion advises that clinicians should monitor CD patients for CVD. How could this be done in practice?

DONE: We have now edited the text to make it clear what steps clinicians can take to improve the management of cardiovascular disease risk among patients with CD.

The text (lines 411-415) now reads: 'clinicians should make their CD patients aware of their elevated risk, advise their patient on lifestyle changes to reduce their cardiovascular risk (including diet, physical activity and weight management), and ensure cardio-protective medications are used (in accordance with national guidelines) to further address major cardiovascular risk factors.'

The authors believe that this is the first such study to employ a CVD risk score and I think that they are correct in this assumption. They were also able to assess risk against length of CD diagnosis which I think is novel.
Health Care workers who look after those with CD need to be aware that CVD is elevated in CD so that optimum treatment can be given and especially preventive measures instituted.
Since many CD patients are now looked after by GPs, nurses or dieticians rather than in gastroenterology clinics, publication of this work in a general journal would seem appropriate.
I believe the information presented is reliable and of interest to all working in the CD field. It should

spur further research in this interesting aspect of CD.

Reviewer: 2

Recommendation:

Comments:
I am not happy with this. It is based in part on questionnaire. CD is self-reported and they only ask about wheat:

Further, a gluten free diet was not taken into account as the diet questionnaire only asked if a wheat free diet was followed (gluten is identified in other grains, such as barley and rye) and the numbers of participants with CD who reported not following a wheat free diet was small, so the impact of a gluten free diet could not be assessed.

Consider this paper (I am not co-author)
Body mass index in celiac disease: beneficial effect of a gluten-free diet
Jianfeng Cheng 1, Pardeep S Brar, Anne R Lee, Peter H R Green

Doesn't seem to have been discussed or cited. The authors also missed key reviews on tis topic.

CD can have difficulties like nutritional deficiency and overweight.

Our definition of CD was not just based on self-reported coeliac disease status (we did not use wheat consumption are part of our case definition), but also incorporated diagnoses captured retrospectively from medical records, as stated in the methods section (lines 124 – 130). There are, however, potential biases associated with both self-reported and inpatient data. This has been expanded on in the discussion:

Lines 373 – 377: "CD status was ascertained using a combination of self-reported and hospital inpatient data. This may lead to an under ascertainment of cases due to participants not self-reporting a diagnosis and not having a diagnosis recorded in their hospital record (due to how diseases are recorded in HES).").

Whilst the diet questions asked of participants at baseline only asked about wheat, participants were asked to report any diseases diagnosed by a doctor, and so the case definition does not only include those that report not eating wheat. We include that as a limitation in our manuscript (Lines 382 – 386), and highlight how it means we cannot accurately assess gluten free diet as a mode of action in the association we have identified. Further, the primary objective of the paper was to investigate the association between CD and CVD and not to explore the role of diet in this relationship.

We have reviewed our references and updated them as appropriate.

Reviewer: 3

Recommendation:

Comments:
The paper adresses a very interesting and challenging issue regardin coeliac disease (CD) and more in general the interaction between immune disorders and cardiovascula disease.
The study is well drawn and the paper comes to fair conclusions .
The numerosity of the population is robust and the analysis accurate.

CD patients have an increased risk of CVD and in particular of cardiovascular events , interestingly no correlation with cerebrovascular disease, infact this pathology is mainly connected with hypertension and in CD patients the prevalence is actually low .

Some minor issues I think authors should address (or stress) in discussion :

1. despite no traditional CVD risk factors seem linked with CD , neither traditional infammatory marker such as PCR, I think it should be stressed in discussion that adhesion to diet prescription is necessary to lower inflammation and the study can not give data about this point , may be other markers (IL-6) may be more efficient in this case

DONE: We have added a line to the discussion to cover that we cannot investigate if adherence to a gluten free diet reduces inflammation and reduces the risk of CVD

Lines 345 – 347: Some studies have shown a gluten free diet to reduce inflammation and CVD in CD patients [11,50], and our study cannot investigate whether adherence to a gluten free diet reduced inflammation and therefore the risk of CVD in CD patients.

2. low adherence to diet (declared 84%) may determine impaired absorption of vitamins (D or E or other) that may influence CVD risk .

DONE: We have added a paragraph to the discussion to cover potential micronutrient deficiencies as a possible reason for the increased risk identified;

Lines 361 – 369: "Another potential mechanism for the increased risk seen is through micronutrient deficiencies. Micronutrient deficiencies (such as vitamins A, B, D and E) have been associated with CVD outcomes, although the evidence is conflicting[53]. Micronutrient deficiencies can occur in CD patients who do not adhere to a gluten free diet (due to malabsorption from villi atrophy), but also occurs in those who do adhere to a gluten free diet, due to the inadequate micronutrients in a gluten free diet[54]. However, micronutrient deficiencies are common in the UK population (especially amongst women)[55,56], and so is unlikely to fully explain the increased risk of CVD seen in CD participants identified in this study."

3. wheat free diet may have increased level of saturated fat or fat and salt and this aspect should be considered in discussion

This is already included in the discussion (Lines 352 – 454)

Reviewer: 4

Recommendation:

Comments:
Strengths of the study: prospective design, large study cohort, results have been adjusted with cardiovascular risk factors, application of cardiovascular risk score.
However, previous knowledge/studies should be cited better. There is already one previous UK Biobank study on celiac disease showing increased risk for cardiovascular diseases (CVD) and increased risk for cardiovascular mortality in patients with celiac disease (Schneider CV, Kleinjans M, Fromme M, Schneider KM, Strnad P. Phenome-wide association study in adult coeliac disease: role of HLA subtype. Aliment Pharmacol Ther. 2021 Feb;53(4):510-518.). Furthermore, recent study on the association between celiac disease and CVD with data adjusted for common cardiovascular risk factors is missing (Naaraayan A, Nimkar A, Jesmajian S, Gitler B, Acharya P Atherosclerotic Cardiovascular Disease Prevalence Among Patients With Celiac Disease in the United States: An Observational Study. Mayo Clin Proc. 2021 Mar;96(3):666-676.). In addition, previous findings of professor Ludvigsson's group from Sweden on celiac disease and CVD should be acknowledged better. Their recent large JAMA study (ref no 16; JAMA 2020 Apr 7;323(13):1277-1285) with 50.000 celiac disease patients showed that individuals with celiac disease were at increased risk of death from cardiovascular disease. And they have previously shown (ref no 12, Aliment Pharmacol Ther 2013 May;37(9):905-14) that despite evidence of an increased risk of ischemic heart disease and higher cardiovascular mortality, patients with celiac disease with ischemic heart disease have a more favorable cardiac risk profile compared with ischemic heart disease in reference individuals.

The paper could be shortened, and text condensed (especially in the discussion; first paragraph repeats the findings, it has no references).

References: some references are given twice (e.g no 12 and 39 as well as 15 and 35 are the same). Some references could be omitted (e.g not so many general review articles, or studies on the incidence and prevalence of celiac disease are needed here). References no 25 and 26 are inadequate.

DONE: We have shortened the discussion and reworded the comparison to other studies section. We have reviewed our references and updated them and ensured no duplication.

Table 1: wheat-free diet: Wording "gluten-free diet" would be correct (celiac disease patients are on a gluten-free diet, they are not avoiding only wheat).

Done: Table 1 states "wheat free diet" as this is the information UKB collected. They did not collect data on gluten free diet until later in follow up, and only for a subset of the cohort. This is stated in the limitations section (lines 382 – 386). We have added a footnote to table 1 to clarify that UKB asked for wheat-free diet and not gluten-free diet.

Table 2 and text in the results:" Celiac disease patients were diagnosed less likely with diabetes". according to several studies and meta-analyses, patients with celiac disease have increased risk for type 1 diabetes mellitus and patients with type 1 diabetes have increased risk for celiac disease. The situation is different in type 2 diabetes. It would be better to give data separately on type 1 and type 2 diabetes mellitus.

We have now sub-typed diabetes into Type 1, 2 and unspecified. Due to the data collected by UKB, this is complex to do (see https://doi.org/10.1371/journal.pone.0162388 for more details). Due to the small number of participants with Type 1 diabetes, we have had to continue using the composite phenotype for the analysis, but we have provided the summary statistics for the sub-types in Table 2 and Supplementary Table 5, which shows the difference risk by sub-type. Thank you for this suggestion. We have also added the following to the text:

Lines 157 – 160: "Further, diabetes was sub-typed into type 1, 2 and unspecified as the association between CD and diabetes is type specific (see supplementary methods and Supplementary Table 1). For the prospective analysis, the composite definition was used due to small numbers of participants with Type 1 diabetes."

Lines 234 – 235 "less likely to be diagnosed with type 2 diabetes, more likely to be diagnosed with type 1 diabetes"

VERSION 2 – REVIEW

REVIEWER 1	Morris, Julie. Competing Interest: None
REVIEW RETURNED	28-Sep-2022

GENERAL COMMENTS	This paper has been revised to take account of many of the issues raised by the reviewers. The subdivision of diabetes has clarified the association with CD, and additional information relating to missing data has been provided. The limitations of the data have been acknowledged appropriately, and the interpretation of results now includes further, useful discussion of the study findings. I have no remaining statistical concerns.