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Peer Review File

Associations between preservative food additives and type 2 diabetes incidence in the NutriNet-Santé prospective cohort

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have sufficiently addressed the comments and concerns raised during the first review. Notably, the quality of the data, robust sensitivity analyses, and much stronger consideration for the public health implications of these results enhance the contribution of this manuscript. Overall, I think it adds to the ongoing discussion around necessary and unnecessary use of additives in the food supply and whether harms exist that can be attributed specifically to additive ingredients rather than nutrient composition alone. The results around preservative food additives extensively used in the food supply- both in UPFs and non-UPFs - increasing T2DM risk is noteworthy, and the additional analyses around UPF and the mediating role of preservative food additives on T2DM helps further the discussion both on UPFs and non-UPFs and factors beyond nutritional composition that may be adversely affecting health. The supporting mechanism revisions also enhanced the clarity of the manuscript.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
The authors have addressed most aspects of the reviewer's comment; however, the approach used for the "negative outcome control" does not meet the methodological purpose of such an analysis. In the manuscript, the so-called negative outcome was created by assigning the same number of incident cases as in the main analysis, but distributed randomly across the cohort in terms of individuals and dates of diagnosis. While this approach can test whether the statistical pipeline produces false positives by chance, it does not function as a negative outcome control for unmeasured confounding.

A negative outcome control should (i) have no plausible causal relationship with the exposure (e.g., by a priori), and (ii) share a similar structure of measured and unmeasured confounding as the primary outcome. Because a random outcome is, by construction, unrelated to all covariates, it cannot be used to evaluate potential bias from unmeasured confounding.

The authors should replace or supplement the random-outcome analysis with one or more prespecified negative outcomes that are biologically unrelated to preservative exposure but likely share key unmeasured determinants and that can be analyzed using the same cohort design and modeling strategy.

(Remarks on code availability)
NA

Reviewer #3

(Remarks to the Author)
The additional information provided after the reviewers' comments has improved the manuscript. The authors have

adequately addressed all comments from reviewer 1.

Some additional comments:
- This is a large observational study with a dietary method well suited for measuring preservatives. However, the number of T2D cases is rather limited. Is this due to the age range of the population or the challenges in measuring T2D?
- Therefore, please provide the age range of the study population.
- Line 418: clarify whether there are more factors than composition and structure
- Abstract: Please clarify how many of the exposures showed a statistically significant increased risk and whether the estimates were adjusted for multiple comparisons. How many preservatives were included in the analyses?
- The authors should more clearly emphasize in both the discussion and the conclusion that the findings are observational and do not support causal interpretation.

(Remarks on code availability)

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
Thanks for addressing the comment. I have no further comments.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
The authors have answered all my comments.

(Remarks on code availability)

AUTHOR'S RESPONSE TO REVIEWERS

Reviewer #1 (Remarks to the Author):

Overall comments

The authors examined the association of food preservatives with the incidence of type 2 diabetes in a French cohort of ~108K participants reporting that intakes of specific preservative additives were positively associated with diabetes incidence in adjusted analyses. This topic is highly relevant to the current debates around food processing. The findings could add to the evidence base that may have the potential to be useful to policy makers to address the issue of whether food additives may help to explain mechanisms of association between ultra-processed foods (UPFs) and health outcomes. The latter is important because some countries have not taken policy action on UPFs partly because mechanisms of health harms have not been clear.

The authors are to be congratulated for their extensive effort in the quantification of preservative intakes derived from repeated 24hr recalls using multiple time-dependent sources, providing what is probably the best individual level preservative data to date. The findings were also robust against a series of sensitivity analyses. They acknowledged several study limitations and strengths. However, some other limitations should be addressed, particularly to improve the methodological rigour and interpretation of results.

→ We thank Reviewer#1 for their positive, insightful, and thorough feedback and for acknowledging the strengths of our results. Reviewer#1's comments are addressed below.

Major points

1. In the introduction, it is important to explain the context that food additives are a large group, briefly describing the main types, of which preservatives are one type. It is relevant to also explain and cite their previous work that reported findings on each of emulsifiers, artificial sweeteners, nitrites/nitrates and on mixtures of food additives, but that now they focus on preservatives more specifically. This will help to set the context out more clearly.

→ We added the following paragraph to the very beginning of the introduction page 4: "*Food additives encompass a wide range of substances which can be divided into different categories based on their functional properties, e.g. colours, preservatives, sweeteners, emulsifiers, etc. Our group already investigated artificial sweeteners[1], emulsifiers[2], nitrites and nitrates[3], and most recently, food additive mixtures[4] in association with type 2 diabetes incidence in the NutriNet-Santé cohort. The present study focuses on preservative food additives, which are massively used by the food industry globally.*"

2. The authors included many food preservatives of both non-antioxidant and antioxidant types, but it is not clear why some are missing. For example, the FSA and EFSA lists include gamma-tocopherol (E308), delta tocopherol (E309)), 4-Hexylresorcinol (E586), Calcium benzoate (E213), Ethyl p-hydroxybenzoate (E214), Sodium ethyl p-hydroxybenzoate (E215), Methyl p-hydroxybenzoate (E218), and Sodium methyl p-hydroxybenzoate (E219) as approved additives, but these were not included in this study. It would really useful for the future use of this work if it was (more) comprehensive or clarify why these could not be included.

→ All 80 preservative food additives classified as such by the Codex General Standard for Food Additives database[5] or the UK Food Standard Agency[6] were considered in this study. However, the occurrence of some of them is very low in the French/European markets (e.g., only 10 products in the World Open Food Facts database contain E308 and none in France (https://world.openfoodfacts.org/facets/additives/e308-gamme-tocopherol). This was the case for all preservative food additives cited by Reviewer#1, as well as for potassium acetate (E261), calcium acetate (E263), carbon dioxide (E290), sodium lactate (E325), potassium lactate (E326), potassium citrate (E332), calcium citrate (E333), and nitrous oxide (E942). Consequently, the proportion of consumers was null for all these authorised preservative food additives in this study population, which did not allow us to study their association with T2D risk. We have added this precision in the methods page 7 and in the footnote to Table 2 page 34.

3. The authors rightly state that "For some of these additives, corresponding substances are naturally present in foods and beverages (e.g., certain antioxidant vitamins such as vitamin C – ascorbic acid)". They assessed participants' intakes of naturally occurring acetic and citric acids, nitrites, nitrates, and sulphites. They adjusted their models for additives analyses for the intake of the corresponding substance coming from naturally occurring sources. As a different approach, are the authors able to separately study the associations of substances derived from naturally occurring sources, to compare the findings with those from synthetically/industrially added versions? For instance, in their prior publication on nitrates and nitrites the authors did such an analysis which was very helpful (ref 26 in the manuscript). It is particularly of interest because in the current paper it is noted that some of the naturally occurring sources contribute substantially (eg from 1% for tocopherols or 5% for acetates on average, to 17% for citric acid, 29% for ascorbates, and 63% for sulphites).
→ In response to this comment, we have now added Supplementary Table 5– "Associations between intakes of acetic acid, citric acid, nitrates, nitrites, sulphites, vitamin C, and vitamin E from naturally occurring sources and type 2 diabetes incidence among study participants from the NutriNet-Santé cohort, 2009-2023 (n=108,723)". The corresponding analysis has been added in the Methods section page 9. We also added in the Results section: "*Naturally occurring acetic acid and nitrates from drinking water were associated with increased type 2 diabetes incidence, while natural sulphites were associated with lower incidence (Supplementary Table 5).*", page 13.

4. There should be a more in-depth interpretation in the context of the food sources of the preservatives, to understand any potential confounding by the foods itself (rather than the preservative), or potential effects by the food matrix (as briefly mentioned in lines 383-386), given the adjustments performed in the model. Also, given the widespread scientific and public interest in UPFs, it is particularly important to know if most of the preservatives are from ultra-processed foods (UPFs) or not. According to the NOVA definition (Monteiro 2019, Public Health Nutr), non-UPFs may also contain additives that "prolong product duration, protect original properties or prevent proliferation of microorganisms". The lack of clarification on the whether the foods were mostly UPF may lead to misinterpretation of the findings or extrapolation of the results as applicable to only UPFs, where in fact some non-UPFs may also contain preservative additives. Relatedly, in Figure 2: the x-axis lists food groups, some of which are labelled as being processed. It is not clear what criteria were used for that, versus some other groups listed (but not marked as processed) such as confectionary or cakes, pastries, dairy products,

breakfast cereals etc. And is the term "processed" a mixture of NOVA groups 3 and 4 or is it only group 3 or only group 4 (ie ultra-processed)? Greater clarity is needed.

→ To better show the vector foods of each studied additive (beyond contributors to the main preservative groups in Figure 2), we have now added the % contribution of each food group to the intake of each preservative additive in Supplementary Table 2. The food vectors of preservative additives were very diverse, encompassing contrasted nutritional profiles in terms of sugar, salt, fat and fibre content. For instance, for potassium sorbate, 26.3% of the intake came from fruit- and vegetable-based products while 21.6% came from fats and sauces. To limit as much as possible confounding bias linked to nutritional profiles of vector foods, all models were carefully adjusted for energy, saturated fats, sodium, dietary fibre, and sugar intakes. This point has been added to the discussion page 19.

→ We thank Reviewer 1 for their insightful remark regarding UPF: indeed, as per NOVA definition, food preservatives are not necessarily markers of ultra-processing, thus some non-ultra-processed foods can also contain food additive preservatives. We have now provided in the Results section page 11 the proportion of food additive preservative intake coming from ultra-processed foods in this population study: 34.6%. Cox model 2 in Supplementary Table 4 was adjusted for the proportion of UPF in the diet (similar results as the main model).

→ We apologise for the ambiguity in the labelling of the X axis in Figure 2. We had added "processed" in front of food group names that are usually perceived a "raw food" (e.g. fruits and vegetables) to make the reader understand that here we talked about all types of fruits and vegetables including those processed or ultra-processed and not only raw. But it is true for all food groups of the X axis. So we have removed the term "processed" from all labels (except "processed meat", which is an established term, regardless of the NOVA classification) to minimise confusion in any way.

5. Regarding Line 191 – how was energy under- or over- reporting defined? When I looked in the Suppl Method 1, I found this information. It seems that a substantial number of participants were excluded (17.2%; n=23,098) for being under-reporters of energy intake. The rationale for excluding them is not clear, especially as there is an adjustment for energy intake. It would have been useful to see the impact of retaining them, adjusting for it, or approaches such as Winsorisation could be considered.

→ As noted by Reviewer#1, Supplementary Method 1 explains how under- and over-reporting was defined. In the main model, it was important to exclude under-reporters, since they corresponded to participants with invalid / very improbable intakes. Indeed, the method developed by Black[7,8] and Goldberg[9] relies on the hypothesis that the maintenance of a stable body weight requires a balance between energy intake and expenditure. The equations developed by Black account for the reported dietary energy intake, basal metabolic rate (calculated using Schofield's equations)[10], sex, age, height, weight, number of dietary records, physical activity level (PAL), and intra/inter-individual variability. Participants were also asked whether they were following any caloric restrictive diet at the moment of the record; in that case, they were included back in the analyses. Thus, only "true" under-reporters, with incoherent / abnormally low energy intakes declared (generally due to partially filled records or errors in the declaration of food quantities) were excluded. Although their exclusion may limit the generalizability of the findings, it was necessary to avoid important exposure classification bias. This rationale is now explained in Supplementary Method 1.

→ In response to this comment, we have included an additional sensitivity analysis in Supplementary Table 4, based on the main model, but on the entire population without excluding under-reporters (model 10). This analysis provided similar results.

Also, it was not clear how many were over-reporters. The limits set were very generous, such as for fruit, an over-reporter would be someone who reported consuming more than 3kg per day of fruit. How were these limits set?
→ 54 participants were excluded for over-reporting on top of the 23,098 excluded for under-reporting. We have clarified this point in Supplementary Method 1. The thresholds we use enable us to flag implausible dietary records while leaving enough margin for very high (but not impossible) consumers. An intake of 3kg of fruits per day is rare and elevated but not impossible. It corresponds for instance to half a watermelon, a cantaloupe and an apple…These limits we set based on the 99th percentile of energy intake (updated every 10,000 new dietary records added to the cohort). This precision is now available in Supplementary Method 1.

6. Was any approach taken for false discovery?
→ Results were presented with and without adjustment for multiple testing by the False Discovery Rate (FDR) method (Supplementary Table 3 and Supplementary Table 3 footnote [e]). Overall, most results were stable when adjusting for multiple testing. If adjusting for multiple testing decreases type I error, it also increases type II error (risk of false negative) and may lead miss out on existing associations, which is why this adjustment is debated[11]. Besides, our results were supported by mechanistic plausibility, and all detected associations in our study showed HRs >1, strongly suggesting they were not going in random directions (which would have been the case if they were due to chance). We have added some discussion about this point page 21.

Other and minor points
1. The 80 preservative food additives listed in the Codex General Standard for Food Additives database or UK Food Standard Agency were eligible for the present study. Did the inclusion of ad hoc lab assays still leave them with the same total number of additives considered?
→ See our response to Reviewer#1 major point 2.

2. For foods where both lab assay and database sources are available, has any comparison between the preservative contents from assays vs from the databases been done? It would be useful to see how much does assaying the foods improve the estimation compared to only using generic databases.
→ Overall, quantitative dose data from ad hoc assays and from databases were similar in magnitude (e.g., for food additive potassium sorbate (E202) in the brand-specific pre-packed carrot salad that we selected: laboratory assay = 0.987mg/100g vs 1mg/100g in the EFSA database for the corresponding generic food item), which was not surprising since EFSA doses correspond to an average of laboratory assay data received by the Agency from EU member states and various contributors. We have added this precision in Supplementary Method 2. In any case, we only attributed a non-null dose (from assays in available, and from generic databases otherwise) to products which actually contained the specific additive, as labelled in its ingredient list.

3. Did the authors consider taking a cumulative average of preservative intake over the duration of the study, given that dietary data were collected every six months?
→ Yes, absolutely, this is what we did in all models: time-dependent cumulative intakes over the whole follow-up period (updated every two years).

4. 16 preservative additives were consumed by at least 10% of the participants and thus were individually investigated in relation with type 2 diabetes incidence; what about

additives consumed by fewer than 10%? What was the choice of 10% based on? In one of their other papers the authors used 5% cut-off.
→ In previous papers based on continuous exposure coding, we indeed considered additives consumed by at least 5% of the population. But here, since RCS indicated non-linear associations for several models, the 3-category coding was retained (tertiles or 0 / <median / >median), which allows less degrees of freedom. With a lower threshold, e.g., 5%, there were barely any incident cases in some categories for some food additives with low consumption rate, and models were not converging. This is why we had to restrain the analyses of individual preservative additives to those consumed by at least 10% of the population study. However, all additives (even those consumed by <10%) were accounted for in sums of categories of preservatives and total food additive preservative intake. We have clarified this point in the Results page 10.

5. How were vitamin C and E supplement use assessed and handled in the analyses?
→ We have included an additional sensitivity analysis in Supplementary Table 4 (model 5), adjusting for baseline supplement intakes of vitamins C and E (continuous, mg), which provided similar results.

6. A thoughtful approach was taken to defining categories of additives, applying two alternative approaches. Did the authors additionally consider presenting results on a continuous scale, such as per standard deviation of the preservative food additive exposures? Not essential but could provide a standardised comparative framework.
→ We indeed first thought to present results on a continuous scale per standard deviation of the exposure or per standardised increment. However, in regards to the Restricted Cubic Splines (RCS) presented in Supplementary Figure 3, many p-values for non-linearity were <0.05 and splines suggested plateau effects for several preservative additives. Thus, the log-linearity hypothesis of a continuous model was not satisfied. We therefore decided to present exposure per categories as defined in the Methods section.

7. Could be helpful to have a flowchart of the N of unique foods recorded from the 24HDRs, of which how many are considered as "industrial food items" (and therefore contain preservatives and its preservative content needs to be assessed), followed by the food-additive pair Ns with preservative content estimated by laboratory assays, EFSA, and GFSA. This will help clarify what do the values in of "3122 food additive data", "5149 food additive data" (Supplementary Method2) mean.
→ We have added the requested flowchart in Supplementary Method 2.

8. Please clarify if covariates like physical activity, smoking, all dietary covariates etc. were baseline only or also time-dependent.
→ Time-dependent variables were used for all dietary data (main exposure and covariates) and baseline data were used for other covariates. This has been clarified in the Statistical Analyses paragraph page 9 and footnotes [c] of Figure 3, [a] of Supplementary Table 3, [a] of Supplementary Table 4.

9. Please clarify the time point at which the time-to-event analysis begins - from beginning of "follow-up" or after completion of first dietary record? Please also clarify if only dietary records before the incidence of type 2 diabetes were included in the estimation of intakes in Supplementary Methods4.
→ Since analyses are based on cumulative time-dependent exposure to food additives measured using all dietary records provided throughout follow-up (and not just enrolment records), the

start of follow-up in the main model was the date of enrolment into the cohort (which corresponds to the completion of the first set of dietary records).
→ We have now also included an additional sensitivity analysis in Supplementary Table 4 (model 9) with follow-up starting at the end of the first 2-year period, which provided similar results.
→ For incident cases, dietary data collected during periods after type 2 diabetes diagnosis were not accounted for (prospective design with exposure preceding the outcome).
We have clarified these points in the Methods page 9 and in Supplementary Method 4.

10. The presentation of both p-trend and likelihood ratio overall p-values in Supplementary Table3 can be confusing for readers, especially when one is significant while the other is not (in addition to the 95% confidence intervals shown for each consumer group). While it has been mentioned in the footnotes, please also clarify in the main Methods section the approach and justification for which tests are used to interpret the results under different linearity conditions, with more details on how p-trend and likelihood ratio p-values were derived, as they directly affect the main results.
→ We agree with Reviewer #1 that this may not be straightforward to understand for the reader. Thus, to avoid any ambiguity, and also because the p-trend is meaningless if the log-linearity assumption is not met, we have removed the double column in Supplementary Table 3, leaving only the retained p-value. We have also clarified the justification and tests used in the two situations, in the Methods section page 9, and in the footnotes to Figure 3 and Supplementary Tables 3, 4, 5 and 6: "*When the log-linearity assumption was not rejected (p for non-linearity ≥ 0.05 in the Restricted Cubic Splines models), the p for linear trend was retained (obtained by coding the exposure as an ordinal categorical variable 1, 2, 3). When the assumption of log-linearity was not met (p for non-linearity <0.05), it was not adapted to calculate a p for linear trend, thus, the likelihood ratio overall p-value was retained (obtained by coding the exposure as a non-ordinal categorical variable and calculating likelihood ratio test between models with and without the studied food additive exposure variable).*"

11. If the authors wish to present both p-trend and likelihood ratio overall p-values in Supplementary Table3, it may be worth commenting on the interpretation of cases where p-trend is not significant, likelihood ratio p-value is significant, but p-trend is used for interpretation due to the no evidence of non-linearity (e.g. Sulphur Dioxide E220, Total Nitrates, Potassium Nitrate E252), or any other similar discrepancies. Could there also be the possibility of lack of statistical power to detect a non-linear association?
→ Please see our answer to previous point -> no longer applicable: to avoid any ambiguity, and also because the p-trend is meaningless if the log-linearity assumption is not met, we have removed the double column in Supplementary Table 3, leaving only the retained p-value.

12. The authors may wish to further discuss the generalisability of the findings to the population of France or particular regions. Did the study collecting data electronically bias the sample to only those able to engage digitally? How representative was the sample (80% were women; was ethnic distribution known, for instance)?
→ Indeed, as in other studies investigating health and diet in which people enrol voluntarily, this study included more women, with a higher educational level and healthier lifestyles than the general French population[12,13]. Therefore, caution is needed in generalising the findings. However, daily energy intake as well as proportion of energy by ultra-processed foods were similar in our population study compared to estimates from French nationally representative surveys, supporting the generalisability of our findings[14,15]. Ethnic representation race/ethnicity and religion were not available in the cohort due to a very restrictive ethical/legal regulation

policy regarding the collection of these data in French epidemiological studies (specific authorisations needed). But overall, the geographical distribution of the cohort also matches the one of the general population in mainland France[16]. Nearly 95% of the French population has access to the Internet[17] and we have shown that the study population was not limited to digitally fluent individuals (with about a quarter of participants reporting being inexperienced in terms of computer use)[18]. It has also been suggested that the use of the Internet reduces social desirability bias[19], which may enhance the quality of the data collected but also provide access to populations more difficult to reach otherwise. For instance, the proportion of unemployed people or beneficiary of public financial allowances was 6.4% in the cohort, which is lower than the about 10% in the general population, but is higher than what is obtained in most population-based studies using traditional methods of recruitment. A population study may not be representative of the target population, this does not automatically result in biased estimates of associations between exposures and outcomes and in aetiological studies, the diversity of the population is more important than the representativeness itself. In NutriNet-Santé, we aimed to have enough contrast between different categories of dietary exposures to conduct aetiological analyses, while accounting for a wide diversity of lifestyle profiles. The Discussion paragraph on the strengths and limitations has been extended to include these elements page 19-20.

13. Line 116 – what is/are "EFAS opinions report"?
→ We clarified the phrasing page 4 regarding the European Food Safety Authority (EFSA): "EFSA suggested that…".

14. Line 148 – how extensive is the NutriNet-Sante' study's food composition table compared to the French national ones?
→ NutriNet-Santé food composition table integrated all available data from the French national composition database (CIQUAL[20]), and further added information on additional components (e.g., food additives, *trans* fatty acids, etc.). This has been added in the Methods page 6.

15. Lines 160 and 414-15: ad-hoc laboratory assays in food matrices were mentioned but no information is provided. Can refer to suppl materials.
→ Indeed, Supplementary Method 2 details information about this point, we refer to it at the beginning of the paragraph "Preservative Food Additive Intakes" page 6.

16. Line 180 related: could clarify here or in discussion section that biochemical assessment was not part of the diabetes case ascertainment.
→ We have clarified in the Methods section page 8 that we did not perform ad hoc biochemical assessment for diabetes case ascertainment.

17. Line 183 – probably biannual means six monthly but I have seen this confused with biennial previously (ie every two years). It may be worth spelling this out.
→ We indeed meant six-monthly, it is now spelt out for clarification.

18. Line 225 – on average participants completed 21 recalls (SD 18); the abstract states up to 84. Should be reconciled, or suggest drop information from abstract unless median and range or IQR included.
→ We deleted the number in the abstract due to strict word constraint, and added a descriptive summary of the distribution of the number of dietary records completed by participants in the Results section, page 10.

19. Line 210: Should be Supplementary Methods4 instead of Supplementary Methods3?

→ Apologies, it is now corrected page 9.

20. Line 273: should be Figure 3 instead of Figure 2?
→ Apologies, it is now corrected page 12.

21. Figure 3: Consider enlarging the text (HRs and additive names) and symbols (* and §) to improve readability.
→ Texts and symbols have now been enlarged in Figure 3

22. Supplementary Table 5: is the EFSA estimate all population or consumers only? Please also state if they are Mean (SD) or otherwise.
→ We have now clarified in the corresponding heading of Supplementary Table 7 that these estimates correspond to all (adult) population, minimum-maximum of mean values across considered dietary surveys. SD were not available in the EFSA surveys[21–43].

23. Line 326 – should provide reference #26 to substantiate the point Indeed, our group previously published a specific study on nitrites and nitrates and type 2 diabetes incidence in NutriNet-Santé.
→ The reference is now added page 15.

24. Lines 360-62 and 376-78 and elsewhere in the discussion: Please check grammar / sentence structure
→ We checked grammar and sentence structure throughout the manuscript.

25. Line 441-43: the concluding statement text states "supports recommendations for consumers and patients to favour minimally processed foods, and limit superfluous additives". The current study did not include patients.
→ We deleted "and patients" in the conclusion page 22.

Reviewer #2 (Remarks to the Author):

Overall, this study found a consistent, adverse associations between numerous preservative food additives and type 2 diabetes incidence, robust to adjustment for numerous confounding variables using highly sophisticated and detailed dietary databases and analyses. The analyses and results presented are both novel and robust, and the authors' conclusions are generally well supported. Overall, this is an important, high quality scientific contribution.
→ We thank Reviewer#2 for their positive and constructing feedback. All comments have been addressed below.

Some suggestions for further improvement are below:
1. Given the ongoing discussions about the role of UPFs in chronic disease risk, more discussion of any differences between model 1 vs. model 2 could be discussed- results were robust to adjustment for %UPF along with other confounding variables, and it would be helpful if the authors discussed the extent to which that supports arguments that the mechanisms linking UPFs with adverse health are driven by the presence of additives more so than other nutrient-based mechanisms.
→ As explained in our response to Reviewer#1 Point 4, as per NOVA definition, food preservatives are not necessarily markers of ultra-processing (unlike other food additives such as artificial sweeteners or colours), thus some non-ultra-processed foods can also contain food

additive preservatives. We have now provided in the Results section page 11 the proportion of food additive preservative intake coming from ultra-processed foods in this population study: 34.6%. This probably contributed to the fact that the results were still statistically significant after adjustment for the proportion of UPF in the diet (model 2 in Supplementary Table 4). This is not incompatible with the fact that some of these additives could contribute to explain part of the association between UPF and elevated risk of type 2 diabetes[45,46]. We have added this point in the Discussion pages 18-19.

→ In response to this comment and following ones, we have also performed a mediation analysis. In this population study, ultra-processed food exposure (% weight in the diet) was associated with higher type 2 diabetes incidence (HR=1.20, 95%CI [1.11-1.29], p-value <0.001). We computed a mediation analysis to assess the proportion of this association mediated by preservative food additives that were related to type 2 diabetes in this study (i.e., potassium sorbate (E202), potassium metabisulphite (E224), sodium nitrite (E250), acetic acid (E260), sodium acetates (E262), calcium propionate (E282), sodium ascorbate (E301), tocopherol-rich extract (E306), alpha-tocopherol (E307), sodium erythorbate (E316), citric acid (E330), phosphoric acid (E338), and extracts of rosemary (E392)). In all, 17% of the association between ultra-processed food and type 2 diabetes was mediated by exposure to these preservatives (p-value of the mediated proportion = 0.01). Information on the method of this mediation analysis has been added to the Methods section page 9 and results are now displayed page 14.

2. Adjustment for overall diet quality (vs. intake of individual food groups) may be informative.

→ In response to this comment, we have included an additional sensitivity analysis in Supplementary Table 4 (model 7), adjusting for overall diet quality represented by Healthy and Western dietary patterns derived by principal component analysis (instead of individual food groups). This model provided similar results. Supplementary Method 5 presents the method for determining these dietary patterns.

3. What methods did the authors use to adjust for multiple comparisons?

→ Please see our response to Reviewer#1, point 6

4. I think more nuanced discussion of how to translate these results into healthy dietary patterns would be helpful. In the conclusions, the authors state the broad advice to reduce UPFs and increase minimally-processed foods, but based on the results and Figure 2, more nuanced guidance could help organize the findings (e.g., to reduce intake of x, y, z, consumers may want to prioritize reducing primary sources of preservative food additives - especially those foods that do not contribute toward nutrients of public health concern - like alcohol, processed meats, etc. I really like Figure 2, but it's difficult to read in grayscale, so if there is anything else that could be done to improve readability, that would be helpful.

In order to comply with this comment and with point 6 below:

→ We have modified Figure 2 to improve readability: the contribution proportion is now reflected by both size and colour of each point (so readable even in greyscale).

→ We have included a figure summarising potential modes of action of food additive preservatives, as suggested by experimental research (Supplementary Figure 4) and shortened the "mechanistic plausibility" section pages 16-17.

→ We have expanded instead the discussion pages 18-19 regarding public health implications: "*In terms of public health implications, these results suggest that a reduction in food additive preservative exposure might beneficiate to type 2 diabetes prevention. As per NOVA definition,*

food preservatives are not necessarily markers of ultra-processing (unlike other food additives such as artificial sweeteners or colours). The proportion of additive preservative from ultra-processed foods in this population study was 34.6%. This probably contributed to the fact that results were still statistically significant after adjustment for the proportion of UPF in the diet. However, this is not incompatible with the fact that some of these additives could contribute to explain part of the association between UPF and elevated risk of type 2 diabetes[45,46]*. In practice, to reduce exposure to certain food additive preservatives such as sulphites or nitrites, for which the primary sources are specific food groups that, in addition, have no particular nutritional value (i.e. alcoholic beverages and processed meats, respectively), it is advisable to limit consumption of these food/beverage groups. However, several preservative food additives (e.g. potassium sorbate, calcium propionate, erythorbates) are ubiquitously used across many food groups, with a huge variability in ingredient list depending on the brands for a same generic food item. This has two public health implications. First, in terms of recommendations to the public, this leads to the formulation of a general guideline aimed at limiting unnecessary preservative additives (choosing preservative-free alternatives whenever possible). This applies to all food groups, including processed and ultra-processed fruit and vegetables. For example, cooking at home and consuming only unprocessed or minimally processed fruit and vegetables would avoid around 25% of total food preservatives. Second, this reinforces the idea that measures targeting individuals (such as disseminating recommendations) will not be sufficient and that policy actions must be implemented in parallel to deeply transform the food supply and reduce exposure. This includes, for example, re-evaluating these additives and, if necessary, amending regulations on authorised substances and doses in order to better protect the population.*"

→ Please also see our response to Reviewer#2 Point 1 on mediation analyses.

5. There are a handful of typos (e.g. p14, line 342, and p18, line 421).

→ Sorry for these typos. We have carefully checked spelling and grammar throughout the manuscript and made appropriate corrections. Of note, some "typos" may come from the difference in spelling in UK and US English (e.g., enrol vs enroll). We have used UK spelling throughout but can adapt if needed, to fit the Journal editorial policy.

6. I like the section on mechanistic plausibility, but believe it is excessive within the broader manuscript. First, I think it would be helpful to significantly condense that section by translating some of the information into a figure. Then, I think more room could be devoted to the public health implications of this research - both toward interim food-based guidance as more extensive re-evaluation of food additives is conducted, and clearer delineation of what this adds to the broader discussion on UPFs and mechanisms linking them to disease. Given that many food groups contain multiple additives, translating some of these results toward more specific dietary guidance using simulation could be a nice addition (e.g., replacing 1 serving of processed meat with a minimally processed meat, would reduce total preservative amount by x which was associated with lower T2DM risk in this sample).

→ Please see our response to Reviewer#2 points 1 and 4.

Reviewer #3 (Remarks to the Author):

General Comments: This study addresses an important public health question regarding the potential impact of food additive exposure on the risk of type 2 diabetes. The use of a large cohort, repeated 24-hour dietary records, and ad hoc laboratory assays of food

matrices are notable strengths. However, several methodological challenges limit the interpretability of the findings. Chief among these is the potential for substantial measurement error in the assessment of food additives and a lack of validation against biological markers. Additionally, since food additives are predominantly found in processed foods, it is difficult to determine whether the observed associations reflect the effects of the additives themselves, the processed foods in which they are found, or overall poor dietary quality. The analytic approach, which relies on time-dependent cause-specific Cox models, may not adequately address time-varying confounding or allow for causal interpretation. Consideration of negative outcome controls could further strengthen the assessment of residual confounding. Specific concerns and recommendations are outlined below.

→ We thank Reviewer#3 for their overall positive comment and for their constructive suggestions which we have taken into account and which have contributed to improve the manuscript (see below).

Specific Comments

1.Validation of Food Additive Exposure Estimates

A major limitation of estimating food additive exposure using dietary assessment tools combined with food additive databases is the risk of substantial measurement error. This stems from the inherent limitations of self-reported dietary data and the fact that additive exposure is estimated from multiple food items, often without accounting for preparation methods (e.g., raw vs. cooked). This methodological limitation parallels long-standing concerns in studying the health effects of nutrients, where nutrient intake estimates based on similar tools have often yielded findings that were not replicated in subsequent randomized controlled trials. While randomized trials on food additives are difficult to conduct, the manuscript should describe whether any validation studies have assessed the dietary estimates against biological markers (e.g., urinary or plasma concentrations of selected additives). If such studies are unavailable, the absence of validation should be clearly acknowledged as a limitation.

→ Although classification bias cannot be ruled out, the estimation of dietary intakes (nutrients and food additives) in NutriNet-Santé are among the most accurate globally in a cohort study (and regarding food additives, the only one). This is due to the high level of the 24h dietary records used (3500 generic foods, including the distinction between raw vs. cooked, organic vs. conventional etc. + commercial brands), and their update during follow-up. Dietary records were validated against interview by a trained dietitian and against blood and urinary biomarkers for energy and key nutrients, showing the robustness of the tool, as previously published[47–49]. A limitation that should be acknowledged is that specific exposure to preservative food additives was not part of these validation studies. Indeed, validation of additive exposure versus plasmatic or urinary biomarkers would be ideal, however, it can be challenging, and even impossible for most food additives, including most preservatives, due to the lack of measurable biomarkers, since most additives are metabolised into substances that are non-specific and/or ubiquitous in human biofluids. Nevertheless, the accuracy of the qualitative and quantitative food additive exposure assessment of the NutriNet-Santé cohort represents an important strength of the study, thanks to detailed and repeated 24h-dietary records, linked to multiple food composition databases (OQALI, Open Food Facts, GNPD, EFSA, and GSFA), ad-hoc laboratory assays in food matrices, and dynamic matching to account for reformulations of industrial food items over time (more information on this process has been added as requested by Reviewer #1 point 7). Besides, intakes of food additive preservatives in this study mostly aligned with EFSA estimates as reported in Supplementary Table 7. We have added these elements in the limitation section of the Discussion, page 20.

2.Challenges in Causal Inference for Food Additives

Estimating the causal effects of food additives is inherently challenging. Ideally, a randomized trial would compare otherwise identical foods differing only in their additive content. In this observational study, adjustment was made for intake of red and processed red meat; however, such adjustment is likely insufficient, as food additives are predominantly found in processed foods. For example, individuals with high additive exposure are likely those who consume more processed foods, whereas those with low exposure tend to consume more fresh or minimally processed foods. Numerous studies have shown that processed food consumption is associated with a higher risk of type 2 diabetes, making it difficult to disentangle the effects of the additives themselves from those of an overall poor diet, processed food consumption, or other components of processed foods (e.g., BPA from packaging, trans fats). From a public health and guideline perspective, it may be more actionable to recommend reducing processed food intake broadly rather than avoiding specific additives within processed foods.

→ We agree with Reviewer #3 on the challenging character of estimating the causal effects of food additives. Of course, RCT would be very useful, but long-term clinical trials on hard endpoints are not feasible for obvious ethical reasons, when deleterious effects are expected. In this case, triangulation of multiple study designs has proven useful to show causality by combining well conducted large scale observational and mechanistic epidemiological studies on long term health outcomes, with short term trials studying intermediate metabolic endpoints, and with *in vivo* and *in vitro* experimental studies. The present paper provides one original, and so far unique, piece of evidence to contribute to this overall picture.

We are well aware of the association between ultra-processed food intake and elevated risk of type 2 diabetes (we published the first prospective study showing this association[45]). However, as explained in our response to Reviewer #1 point 4: *as per NOVA definition, food preservatives are not necessarily markers of ultra-processing, thus some non-ultra-processed foods can also contain food additive preservatives. We have now provided in the Results section page 11 the proportion of food additive preservative intake coming from ultra-processed foods in this population study: 34.6%. Cox model 2 in Supplementary Table 4 was adjusted for the proportion of UPF in the diet (similar results as the main model). Besides, the food vectors of preservative additives were very diverse, encompassing contrasted nutritional profiles in terms of sugar, salt, fat and fibre content. For instance, for potassium sorbate, 26.3% of the intake came from fruit- and vegetable-based products while 21.6% came from fats and sauces. To limit as much as possible confounding bias linked to nutritional profiles of vector foods, all models were carefully adjusted for energy, saturated fats, sodium, dietary fibre, and sugar intakes.* This point has been added to the discussion page 19.

In order to comply with Reviewer #3's comment, we have included an additional sensitivity analysis in Supplementary Table 4 (model 6), further adjusting our models for *trans* fatty acid intake. Results were similar to those of the main model.

Finally, we fully agree with Reviewer #3 regarding the recommendation to the population. In France, the official guideline from the French National Nutrition and Health Program (that our research team strongly contributed to adopt) has a broader scope than food additive preservatives and is formulated as "favour fresh and minimally processed foods, and limit superfluous additives whenever possible", as stated in the conclusion page 22.

3. Natural vs. Additive Sources

The study reports an association between citric acid and increased risk of type 2 diabetes (HR: 1.29; 95% CI: 1.11–1.51). Since citric acid is naturally abundant in citrus fruits, it would be informative to stratify the analysis by levels of fresh fruit and vegetable intake

to assess whether the association persists among individuals with high consumption of these natural sources.

→ In response to this comment, we have added Supplementary Table 8 showing the associations between citric acid (E330) food additive intakes and type 2 diabetes risk stratified by the naturally-occurring intake of citric acid. The direction and order of magnitude of the HR in the two strata were close (1.20 (0.96 -1.51) and 1.35 (1.09 -1.66) below and above the sex-specific median of naturally-occurring citric acid, respectively), with no interaction detected between naturally occurring and food additive citric acid ($p_{interaction}$ = 0.54).

Besides, as requested by Reviewer #1, point 3, we have now also added Supplementary Table 5, showing associations between intakes of acetic acid, citric acid, nitrates, nitrites, sulphites, vitamin C, and vitamin E from naturally occurring sources and type 2 diabetes incidence. The corresponding analysis has been added in the Methods section page 9. We also added in the Results section: "*Naturally occurring acetic acid and nitrates from drinking water were associated with increased type 2 diabetes incidence, while natural sulphites were associated with lower incidence (Supplementary Table 5).*", page 13.

4. Analytic Approach and Time-Varying Confounding

The study used time-dependent cause-specific Cox models, which, while commonly applied, have notable limitations in the presence of time-varying confounding. First, traditional time-varying Cox models may produce biased estimates when time-varying confounders are influenced by prior exposure. Second, cause-specific hazard estimates generally cannot be interpreted as causal effects because individuals who remain at risk at each follow-up period under high versus low food additive exposure are not comparable (See Young, J.G. et al Statistics in medicine, 39(8), pp.1199-1236). This issue becomes relevant when the competing risk of death is non-negligible—as in this study, where the incidence of both type 2 diabetes and death is approximately 1%. Finally, a strong parametric assumption is made for all covariates. Relaxing modeling assumptions for the covariates are also needed.

→ In response to this comment, we have included an additional sensitivity analysis in Supplementary Table 4 (model 11), re-running all analyses by using marginal structural models, with inverse probability weighting, as recommended in the paper of Young et al.[50] mentioned by Reviewer #2. Results of this model were overall similar to those of our main model, except for total ascorbates that became non-significant with p=0.4, maybe due to loss of statistical power (this model being restricted to participants with at least two periods of follow-up). Of note, the specific ascorbate E301 remained stable with this model (p<0.001). We added this information in the Results section page 13.

→ We also verified that the Schoenfeld residual test was not statistically significant (all p-values > 0.05) for all exposures and adjustment factors, which supports the compliance with the Cox proportional hazard assumption for all covariates (Supplementary Figure 2).

→ Finally, we have added a sensitivity analysis in Supplementary Table 4 (Model 12), relaxing the log-linearity assumption for covariates by adding splines (overall similar results).

5. Use of Negative Outcome Controls

Given the high likelihood of residual and unmeasured confounding, the use of negative outcome controls could strengthen the study. It will be helpful if the study considers some negative outcome control (e.g., certain infection disease, or vaccination uptake).

→ In response to this comment, we have performed an additional analysis (Supplementary Method 4 and Supplementary Table 6) showing associations between food additive preservative exposure and a "random" outcome, i.e. same number of incident cases as in our main analysis,

but distributed randomly across the cohort population study in terms of individuals and dates of diagnosis. As expected, no statistically significant association was observed.

6. Dietary Assessment Frequency and Completeness

The use of three validated 24-hour dietary records every six months is a strength, as it enables the capture of time-varying dietary patterns. It would be helpful to include a descriptive summary of the distribution of the number of dietary records completed by participants.

→ Indeed, participants were invited to complete 3 24HDRs every six months. Since dietary record update was not mandatory, not all participants completed all records (in this case, their intakes of the previous period were kept). Therefore, we used updated intake value for each participant whenever available, making the most of all follow-up data, and systematically adjusting for the number of answered 24HDRs in all models. As also asked by Reviewer#1, we have added a descriptive summary of the distribution of the number of dietary records completed by participants in the Results section, page 10: on average, they completed 21 24HDRs (SD 18); median=17; 25th-75th percentiles=6–32; maximum=84. We also expanded the limitation section on this point page 21.

7. Clarification on Start of Follow-Up

The study includes participants who completed at least two 24-hour dietary records within the first two years of follow-up. It would be helpful to clarify the definition of the follow-up start date and whether inclusion criteria were based on conditions measured after the start of follow-up. This clarification is important for evaluating potential selection bias and temporality.

As stated in our response to Reviewer#1, minor point 9:

→ Since analyses are based on cumulative time-dependent exposure to food additives measured using all dietary records provided throughout follow-up (and not just enrolment records), the start of follow-up in the main model was the date of enrolment into the cohort (which corresponds to the completion of the first set of dietary records).

→ For incident cases, dietary data collected during periods after type 2 diabetes diagnosis were not accounted for (prospective design with exposure preceding the outcome).

We have clarified these points in the Methods page 9 and in Supplementary Method 4.

→ We have now also included an additional sensitivity analysis in Supplementary Table 4 (model 9) with follow-up starting at the end of the first 2-year period, which provided similar results overall.

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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have sufficiently addressed the comments and concerns raised during the first review. Notably, the quality of the data, robust sensitivity analyses, and much stronger consideration for the public health implications of these results enhance the contribution of this manuscript. Overall, I think it adds to the ongoing discussion around necessary and unnecessary use of additives in the food supply and whether harms exist that can be attributed specifically to additive ingredients rather than nutrient composition alone. The results around preservative food additives extensively used in the food supply- both in UPFs and non-UPFs - increasing T2DM risk is noteworthy, and the additional analyses around UPF and the mediating role of preservative food additives on T2DM helps further the discussion both on UPFs and non-UPFs and factors beyond nutritional composition that may be adversely affecting health. The supporting mechanism revisions also enhanced the clarity of the manuscript.
→ We thank Reviewer #1 for their thoughtful and constructive comments as well as their appreciation of our work.

Reviewer #2 (Remarks to the Author):

The authors have addressed most aspects of the reviewer's comment; however, the approach used for the "negative outcome control" does not meet the methodological purpose of such an analysis. In the manuscript, the so-called negative outcome was created by assigning the same number of incident cases as in the main analysis, but distributed randomly across the cohort in terms of individuals and dates of diagnosis. While this approach can test whether the statistical pipeline produces false positives by chance, it does not function as a negative outcome control for unmeasured confounding.

A negative outcome control should (i) have no plausible causal relationship with the exposure (e.g., by a priori), and (ii) share a similar structure of measured and unmeasured confounding as the primary outcome. Because a random outcome is, by construction, unrelated to all covariates, it cannot be used to evaluate potential bias from unmeasured confounding.

The authors should replace or supplement the random-outcome analysis with one or more prespecified negative outcomes that are biologically unrelated to preservative exposure but likely share key unmeasured determinants and that can be analyzed using the same cohort design and modeling strategy.
→ We thank Reviewer #2 for their insightful remark. We have now selected hip fracture as an outcome with no expected causal relationship with studied exposures for the "negative outcome control model". As expected, no statistically significant association was detected (all p-values>0.05). We have modified the Methods section on page 9 and the Results section on page 13 accordingly.

Reviewer #2 (Remarks on code availability):

NA

Reviewer #3 (Remarks to the Author):

The additional information provided after the reviewers' comments has improved the manuscript. The authors have adequately addressed all comments from reviewer 1.
→ We thank Reviewer #3 for their constructive comments. Please see our responses to their questions below.

Some additional comments:
- This is a large observational study with a dietary method well suited for measuring preservatives. However, the number of T2D cases is rather limited. Is this due to the age range of the population or the challenges in measuring T2D?
- Therefore, please provide the age range of the study population.
→ Indeed, this population study did not focus on middle-aged and senior participants, since recruitment started at 15 years old. Participants had a mean age at baseline of 42.5 years (SD 14.6) (range: 15.2-99.0). This is now specified in the manuscript on page 10.
Besides, as in other population-based cohorts investigating health and diet, participants included more women, with a higher educational level and healthier lifestyles than the general French population[1,2]; thus, potentially explaining the observed low rate of ascertained type 2 diabetes (1.04% in this study against 5.6% people being medicated for diabetes in France[54]). Multisource case ascertainment combining participant declaration of a medical diagnosis of type 2 diabetes by a physician with yearly registration of all medication use linked to the Vidal® database and the link with medico-administrative databases limited the risk of not detecting diagnosed cases. Moreover, in France, nationally-representative French data estimated that only 1.7% adults had undiagnosed diabetes[55], limiting the potential bias due to undiagnosed diabetes cases. We have added these elements to the discussion page 21.

- Line 418: clarify whether there are more factors than composition and structure
→ Many parameters play a role; we have now clarified that saying that structure and composition are not the only factors playing a role (page 17): *"The effects of one substance may vary based on factors such as food matrix (of which composition, structure, pH and other characteristics may affect the way bioactive compounds are assimilated and digested by gut microbiota and host organisms),…"*.

- Abstract: Please clarify how many of the exposures showed a statistically significant increased risk and whether the estimates were adjusted for multiple comparisons. How many preservatives were included in the analyses?
→ 17 food additive preservatives were studied individually; 13 of them were significantly associated with an increased risk of type 2 diabetes, and 12 remained so after adjustment for multiple test comparisons. Those precisions are now available in the abstract as requested by Reviewer #3.

- The authors should more clearly emphasize in both the discussion and the conclusion that the findings are observational and do not support causal interpretation.
→ As requested, the discussion (page 19) and conclusion (page 22) now both state that the findings of this study are observational and, by nature, do not allow causal interpretation based on this study alone.

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