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Debras C, Chazelas E, Sellem L, Porcher R, Druesne-Pecollo N, Esseddik Y et al. Artificial sweeteners and risk of cardiovascular diseases: results from the prospective NutriNet-Santé cohort *BMJ* 2022;378:e071204

What We Know, Think We Know, or Are Starting to Know

What we definitely know: artificial sweeteners are divisive. While we also know that artificial sweeteners are beloved targets of Le Quacks, is there a case to argue that they equally overzealously defended by the Evidence-Based Bro's [EBB]?

Could there be a case to answer that artificial sweeteners may in fact pose a risk, or at the very least a case to answer that currently accepted safety thresholds may need revisiting? There are two related questions here: the first is safety, i.e., derived from toxicology research and regulatory assessments* [see **Geek Box**, below]; the second is risk, i.e., human outcome data from population and intervention studies.

Back in July, we took a Deepdive into the first epidemiological study to analyse the effects of replacing sugar-sweetened beverages [SSB] with artificially-sweetened beverages [ASB]; the study showed a 12% lower risk of coronary heart disease when making such a swap. This appears to support the intended purpose of AS in the food supply; to displace added sugar and, consequently, improve health through lowering added sugar intake.

The NutriNet-Santé cohort is a large prospective cohort study of nutrition and health in the French population. And it has recently generated two papers on artificial sweeteners that have blown social media's fickle roof off: one paper suggesting an increased risk of cancer [Danny, Dr Niamh, and myself covered this study in this episode of *Sigma Nutrition Radio*], and one suggesting an increased risk of cardiovascular diseases. Well grab your Diet Coke because the latter, we Deepdive into now…

*Geek Box: Artificial Sweeteners Regulatory Framework

The main artificial sweeteners [AS] currently in use are aspartame, acesulfame-K, saccharin, and sucralose. Each compound is structurally unique, meaning the individual compounds all vary in sweetness potency, duration of sweetness, aftertaste, and mouth feel. These are approved for use in the European Union [EU] through the European Food Standards Agency [EFSA] and in the U.S. by the Food and Drug Administration [FDA], through a process involving submission of both scientific safety evaluation and technical data. 'Technical data' includes the chemical composition of the compound, its source and manufacturing methods, its stability across a range of food matrices, and its sensory properties. 'Safety data' includes the full range of studies on safety, derived from animal toxicology studies, and includes the anticipated daily intake in the population from all dietary sources, within different ages groups. The animal toxicology studies have specific criteria for the design and type of the studies required based on a system of "Concern Levels"; due to their potential high exposure in the population, AS are considered a "high concern" level. Thus, the animal studies [rodents mostly] must assess both absolute toxicity thresholds and also sub-chronic long-term toxicity for potential effects on reproduction, development, carcinogenicity, genotoxicity, and immunotoxicity. These studies are used to establish the "No Observable Adverse Effect Level" [NOAEL]; the Acceptable Daily Intake [ADI] is then established by dividing the NOAEL by an "uncertainty factor" of 100. The potential health impacts from exposure to the compound in the food supply is assessed by combining data on anticipated intake on the concentrations of the AS anticipated for use in food and beverages together with the quantity of those foods/drinks typically consumed. The requirement for this calculation is to combine the maximum permitted level of the compound in foods together with the maximum level of consumption of food/drink. Thus, these processes are highly conservative assessments, particularly for children and the elderly.

The Study

The NutriNet-Santé cohort is a large prospective cohort of French adults participating in a web-based study of nutrition and health outcomes. Participants completed five online questionnaires in relation to diet, lifestyle, physical activity, health, and sociodemographic data; dietary assessment was repeated every 6-months.

The exposures and outcomes of interest for the present analysis were as follows:

- **Exposure**: Total AS from all dietary sources and all AS types, and additionally each of aspartame, acesulfame-K, and sucralose individually. The exposure groups were categorised as:
    - Never Consumers
    - Low Consumers [below the sex-specific median intake in the cohort]
    - High Consumers [above the sex-specific median intake in the cohort]
- **Outcome**: Cardiovascular disease [CVD], categorised as:
    - Overall CVD
    - Coronary heart disease [CHD]
    - Cerebrovascular disease [CVA]
- **Covariates Adjusted for in Analysis**: age, sex, educational level, smoking status, physical activity, family history of CVD, total energy intake, alcohol, sugar, sodium, saturated fatty acids, polyunsaturated fatty acids, fibre, fruit and vegetables, and red and processed meat.

A sensitivity analysis was also conducted in which intakes of AS were divided into quartiles [non-consumers, low, moderate, and high consumers, respectively]. Further sensitivity analyses also analysed the associations relative to those with an average of seven completed 24 h recalls, adjustment for healthy vs. western dietary pattern, weight loss, and social desirability bias scores.

Results: 103,388 participants were included in the analysis, of which 62.9% were 'non-consumers' of AS, 18.5% were 'low consumers', and 18.5% were 'high consumers'. Average follow-up was 9yrs.

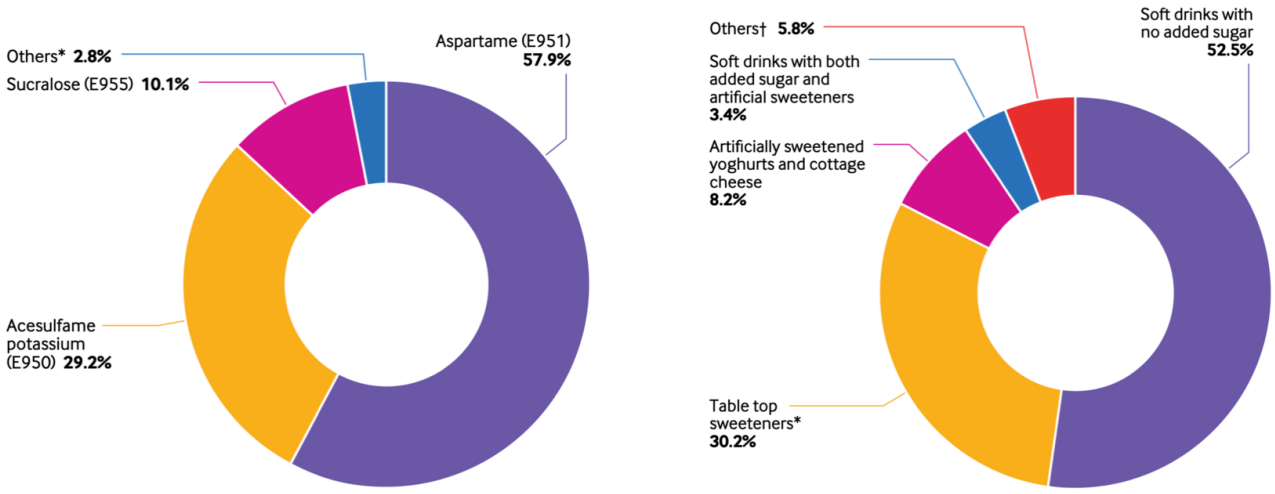

***Figure** from paper showing [**left**] the contributions of each AS to total AS intake, and [**right**] the dietary sources of AS.*

The median intake of total AS in all consumers was 42.4mg/d; average intakes for the low and high consumer categories of total AS were 7.4mg/d and 77.6mg/d, respectively. The average intake in the cohort of 42.4mg/d corresponds to ~100ml of an ASB or one packer of table-top sweetener.

Outcomes

- ***Overall CVD*:** There was a 9% [HR 1.09, 95% CI 1.01 to 1.18] higher risk for total CVD associated with higher [above the median] total AS intakes. The findings for aspartame, acesulfame-K, and sucralose individually, were not significant.
- ***CHD*:** There was no association between total AS intakes and intakes of aspartame and CHD risk. Acesulfame-K was associated with a 40% [HR 1.40, 95% CI 1.06 to 1.84] higher CHD risk, while sucralose was associated with a 31% [HR 1.31, 95% CI 1.00 to 1.71] higher CHD risk. As is evident from the confidence intervals, these are highly imprecise estimates.
- ***CVA*:** Both total AS intake and aspartame were associated with 18% [HR 1.18, 95% CI 1.06 to 1.31] and 17% [HR 1.17, 95% CI 1.03 to 1.33] higher risk of cerebrovascular disease, respectively. Neither acesulfame-K nor sucralose showed any significant associations with CVA. 18.5% were 'low consumers', and 18.5% were 'high consumers'. Average follow-up was 9yrs.

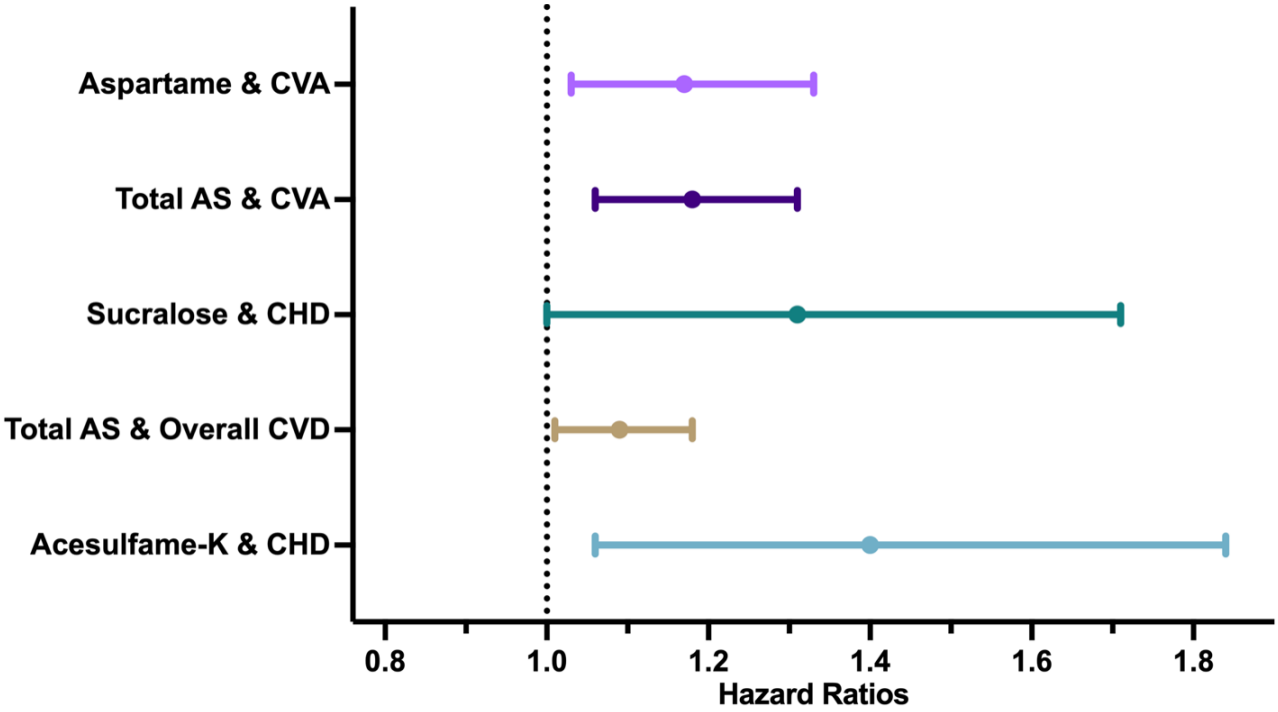

Figure *showing the hazard ratio point estimate [**midline circle**] and 95% confidence intervals [**left and right 'arms' from the circle**] for the statistically significant associations in the present study. As you can see from the left-hand arm of the 95% CI, many of these findings are borderline, and the width of the arms demonstrates a lack of precision, i.e., variability in the data. Nevertheless, the direction of effect is clear for these outcomes and the findings do warrant our attention. One critique we could stand over is, with the lower bound of the 95% CI flirting with 'null', this invites the suggestion that repeating such a study may not necessarily yield significant associations [or even the entirely same direction of effect].*

The Critical Breakdown

Pros: The NutriNet-Santé study is, overall, a well-executed work of nutritional epidemiology. The dietary assessment is more robust than an analysis based on 24 h recalls would imply; a 2yr average was used to determine baseline levels, and 24 h recalls covering two weekdays and one weekend were repeated every 6-months. 24 h recalls were validated against biomarkers and dietitian interviews. Participants had an average of five completed 24 h recalls included in the analysis. A more validated means of determining inclusion based on self-reporting data was used, which uses energy balance estimates to identify under/over-reporters. Levels of aspartame, acesulfame-K and sucralose in food products were quantified by laboratory analysis. The analysis considered AS intakes from all foods and beverages, not just from ASB as previous studies have. The date of consumption of AS-containing food products was matched with the composition data of those food products at the time of the dietary assessments. The statistical analysis was appropriate for this type of study and adjusted for relevant potential confounders, and several informative sensitivity analyses were also conducted. The follow-up period was adequate with a substantial number of person-years follow-up [904,206 person-years, i.e., the cumulative total duration in the study of each participant].

Cons: The paper says that 103,388 participants "were selected" from the total cohort, but no detail is provided on the rationale or method of selection [which may infer some bias]. Most participants [~63%] reported no consumption of AS, which means that the categories of consumption had low overall numbers of participants and very low numbers of incident cases of the outcomes. Thus, the potential for some "false positives" cannot be ruled out given these comparisons were very underpowered. No analysis of food sources to determine whether there was a difference between, e.g., AS-sweetened yogurts vs. diet sodas, was conducted. While the adjustment model was thorough, no adjustment was made for hypertension, which although low in overall numbers was higher in the AS consumers compared to non-consumers. As a predominantly young, female, health-conscious cohort in France, caution should be taken in generalising the findings to other population groups.

Key Characteristic

We need to talk about incident cases, specifically the very low number of outcome events in the present study. Typically, nutritional epidemiology is interested in investigating *incidence* of disease, e.g., how many new cases of CVD or CHD develop in a cohort over a 10yr period.

To understand whether a particular exposure increases risk of disease, epidemiological research compares the event rate in an "exposed group" to the rate in an "unexposed group" (the word exposed is in quotation marks here because technically there is no zero-exposure in human nutrition; standard practice is to rank participants according to levels of intake and compare "high" vs. "low", both of which are also relative concepts!).

All estimates of risk, at their core calculation, include the total number of participants in a group and the total number of events [i.e., incident cases] in that group; thus, these risk estimates are comparing *proportions* between categories. This is important; for example, in the association between acesulfame-K and CHD, the difference in incidence rates between higher consumers and non-consumers was 167 and 164 cases per 100,000 person-years, respectively, yet seemed to produce an enormous risk estimate of 40% higher risk in the higher intake group.

It is important to note that this was not the case across the board; the difference in incidence rates for sucralose was 271 and 161 cases per 100,000 person-years, respectively. But this is where further potential implausibility enters the fray; the highest intake of sucralose was 7.4mg/d. Thus, this analysis compared 7.4mg/d vs. 0mg/d against those respective incidence rates and derived a 31% higher risk with imprecise confidence intervals. 7mg/d; stuff must be lethal, no?

Now, if we look in the supplementary data for total CVD and total AS, in the analysis that divided AS groups into tertiles, the incident cases were 224, 179, and 147 for the low, moderate, and high AS categories, respectively. So, the crude event rate *decreased* as levels of AS *increased*. This is uniform across each of those outcomes when categorising either total AS or individual AS; the incident cases *decrease* as the intake categories *increase*. In this analysis, the hazard ratios are highest in the lowest AS category.

Do we think some component of chance may be at play in these findings, given the low number of events? This is my major amber light for this study; until these findings are replicated in a cohort with much greater numbers of AS consumers *and*, more importantly, greater numbers of endpoint events, I'm not sure we can be confident that the potential for "false positives" can be entirely ruled out.

Interesting Finding

In the substitution analysis, there was no significant association for replacing sugar with AS for any of the outcomes [CVD, CHD, or CVA]. This is counter-intuitive given the known increased risk for CVD associated with added sugar intake [(1)], and the expected benefit to replacing sugars in the diet with non-caloric or low-calorie alternatives.

In the July Deepdive, the 2020 analysis that showed a 12% lower risk of CHD events from replacing SSB with ASB included 280,886 participants followed over 8.2yrs, in which 4,248 events occurred. Thus, both in terms of total sample size and number of events that analysis – over a similar follow-up period to the present study – would have more power to detect more robust associations. A further 2021 paper using data from the U.S. National Health and Nutrition Examination Survey also showed a modest risk reduction of 7% for total mortality and 11% for CHD mortality when replacing one serving of SSB with ASB [(2)].

Now, both aforementioned studies only looked at beverages; the strength of the present study is that it looked at AS intakes from all dietary sources. The findings also challenge the concept, and evidence from studies looking at beverages, that there is no benefit to replacing sugar with AS.

Relevance

This is without doubt the most accurate quantification of AS intake in any epidemiological research to date, and the findings warrant to be taken seriously and not summarily dismissed. The dietary assessment method, the quantification of sweetener levels in foods/beverages, and the validation of the dietary assessment against urinary biomarkers and interviews, is very robust.

Nevertheless, if we give the findings the attention they deserve, it does become difficult to substantiate any biological plausibility to the associations observed. First, from the perspective of the regulatory processes that exist to ensure the safety of food additives entering the food supply, were these findings to be true it would represent a gross failing of the current regulatory frameworks. It would carry enormous implications for the use – and probably herald the end – of animal toxicology models for human populations.

Secondly, it is difficult based on current understanding to understand what mechanism(s) could explain these associations. For example, diet sodas have been associated with higher blood pressure and the sodium content purported to explain this association [3]. But at ~40mg sodium in an average diet soda, this is difficult to reconcile against the low levels of ASB intakes in the present study equivalent to ~100ml/d diet soda.

There are open questions that preclude accepting the validity of these associations, at least for now.

Application to Practice

Remember, if you're even stuck for an explanation, just say: *"microbiome!"*

One wishes that were actually a joke. On a serious note, while AS are not biologically inert, the exposure levels in the present study are orders of magnitude lower than the ADI. So, the first open question from the findings from the NutriNet-Santé studies on AS for now is directed at the regulatory bodies, and frameworks for evidence assessment, to continually demonstrate that these additives are safe at habitually consumed population levels of intake.

The second is more a case of, without reaching for *"microbiome!"*, what do we think may be at play here? My opinion based on the data in this study is that it is likely highly underpowered for the exposure and outcome of interest; the overall numbers of AS consumers was small, the number of events occurring in these groups were smaller again, and the actual levels of AS intakes were even smaller again.

The potential for chance and false positives influencing the outcomes, despite the adjustment for relevant covariates, looms over the outcomes. When we then consider that larger studies with a 3-fold higher numbers of events have not shown these associations, this should give us pause for caution in making conclusions until such findings are repeated in another cohort with more power.

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