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Etemadi A, Sinha R, Ward MH, et al. Mortality from different causes associated with meat, heme iron, nitrates, and nitrites in the NIH-AARP Diet and Health Study: population based cohort study. BMJ. 2017;357:j1957.

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

We know that in 2015, the International Agency for Research on Cancer [IARC] Working Group classified processed meat as "*carcinogenic to humans*", and unprocessed meat as "*probably carcinogenic to humans*" [(1)]. The designation with regard to processed meat is generally accepted in the nutrition community, given that is based on '*consistent associations…in different populations, which make chance, bias, and confounding unlikely as explanations…*' that processed meat causally increases risk for cancer [(1)].

However, the unprocessed meat conclusion was more controversial at the time, primarily because the epidemiological data was unclear. As it was unclear, there was heavy reliance on mechanistic studies, which related to to experimental studies in relation to factors like heme iron, nitrosamines, and heterocyclic amines [HCAs] and polycyclic aromatic hydrocarbons [PCAs] compounds formed from cooking [(1)].

However, the criticism that can arise when mechanistic research fills in the gaps for inconsistent epidemiological findings, is that experimental models do not necessarily extrapolate to the effects of a whole diet in humans. Thus, the effects of not only unprocessed red meat, but potential moderating factors like heme iron and nitrites, have remained inconsistent in the epidemiological data.

While the IARC classification related to cancer, associations have also been observed for other chronic lifestyle diseases of concern, in particular heart disease and type-2 diabetes, and red and processed meat consumption [(2,3)].

The role of red meats, and constituents of meat products in particular [i.e., heme iron, nitrates, nitrites, HCAs, PCAs], remains an important question for nutrition science to address.

The present study investigated red meat, white meat, heme iron, nitrates and nitrites, in relation to all-cause mortality and 9 specific disease outcomes.

The Study

The National Institutes of Health American Association of Retired Persons) Diet and Health [NIH-AARP] prospective cohort study began recruitment in 1995-1996, men and women aged between 50-71yo. Participants were recruited from 6 different states [California, Louisiana, Florida, North Carolina, New Jersey, Pennsylvania] and 2 major metropolitan areas [Atlanta, Georgia; Detroit, Michigan].

Dietary assessment used the National Cancer Institute Diet History Questionnaire, a food-frequency questionnaire [FFQ] with 124 food items, which also included questions specific to regional and ethnic considerations. The initial FFQ was validated against a subgroup of 1,953 participants who had completed the baseline FFQ, and two non-consecutive 24-hour dietary recalls that were separated by a median of 21-days.

The correlation coefficients* were higher in the NIH-AARP for major nutrients than most prospective cohort studies. The NIH-AARP conducted two more calibration studies: one to correct for error in correlations between the baseline FFQ and dietary intake of nitrates and nitrites, and a second to correct for error in estimates of daily red meat intake. The correlation coefficients for nitrates were 0.59 and 0.57, and for nitrites 0.59 and 0.58, in men and women, respectively.

For the present analysis, nutritional variables were divided into quintiles, with the lowest fifth of intake as the reference category. Levels of intake in the highest fifth were compared against the reference category. Variables were also analysed per 20g/1000kcal/day increments. The investigators for the present study conducted a substation analysis, which models the the effects of replacing red meat with white meat. For heme iron, nitrates, and nitrites, mediation analysis was conducted, which explores the extent to which the associations between the exposure [red meats] and outcomes [disease] can be explained by these meat constituents.

The fully adjusted model included sex, age at entry to study, marital status, ethnicity, education, socioeconomic status, history of disease and baseline disease, smoking status, body mass index, physical activity, alcohol intake, and fruit and vegetable intake.

The primary outcomes were the associations between red meats, white meats, and meat constituents heme iron, nitrates, and nitrites, on 9 disease mortality outcomes.

While the study reported on 9 mortality outcomes, results are presented here for the main cardiometabolic diseases [heart disease, stroke, diabetes], and cancer.

*Geek Box: Correlation Coefficients

The correlation coefficient, also known as the 'coefficient of variation' [CV] is the result of a statistical analysis of the results of the food-frequency questionnaire used in a study compared to the results from the validation study for that FFQ. The most robust validation occurs when 7-day measured food records are kept by a random sample of participants in the cohort; this provides a more objective assessment of diet to compare the accuracy of the answers from participants in the FFQ. The result of these correlation analysis is a score, essentially a percentage. For example, if the CV for fibre was 0.58, that would indicate that the FFQ correlated to the measured food records by 58%. A good paper in nutritional epidemiology will state what the CV for the foods or nutrients of interest was, giving an idea of how the findings may relate to a more 'true' level of intake of the exposure of interest. The CV varies across dietary constituents. The CV for fat and carbohydrate in most major cohort studies is >0.60, while the CV for micronutrients, for example zinc, is often <0.50. The CV is something to think about in assessing prospective cohort studies, because if the exposure of interest has a low CV [for example, <0.50], then it may indicate that the particular nutrient is prone to measurement error in that cohort. It would yield a degree of caution in interpreting the findings. Conversely, a CV of >0.60 would indicate a good level of correlation, that has sufficient granularity to capture a relationship between the exposure and the outcome. In the NIH-AARP, the CV for carbohydrates, fibre, fat, and saturated fat, were all >0.70.

Results: 536,969 participants [316,505 men and 220,464 women] were included in the analysis. Over 7.5-million person-years of follow-up, with a median follow-up period of 15.6yrs, 128,524 people died (84 848 men and 43 676 women).

The range of red meat intake was from the lowest of 9.3g/1000kcal to highest of 62.5g/1000kcal [i.e., 156.25g/d for a 2,500kcal/d diet]. Processed meat ranged from 5.1g/1000kcal to 19.4g/1000kcal.

Participants with higher red meat consumption were more likely to be: male; non-Hispanic white; current smokers; have a diagnosis of diabetes; poor to fair perception of their health status; less physical activity; lower socioeconomic status scores; lower attained education levels; lower fruit and vegetable intake; higher BMI; higher energy intake. All of these variables were adjusted for in the fully adjusted model, thus the results presented here survived adjustment for these potential confounders*.

Comparing the highest intake of total [processed and unprocessed] red meat with the lowest, the following findings were observed [pay attention to the confidence intervals]:

- All-cause mortality: 26% increase in risk [HR 1.26, 95% CI 1.23-1.29]
- Cancer: 18% increase in risk [HR 1.18, 95% CI 1.14-1.23]
- Heart disease: 27% increase in risk [HR 1.27, 95% CI 1.22-1.33]
- Stroke: 17% increase in risk [HR 1.17, 95% CI 1.05-1.30]
- Diabetes: 44% increase in risk [HR 1.44, 95% CI 1.26-1.64]

The increase in risk was linear across quintiles of intake. When analysed separately as unprocessed and processed meat, the results were similar. Analysing intakes per 20g/1000kcal/day increases in either unprocessed or processed meat also yielded similar results.

White meat was associated with significant reductions in risk, comparing the highest to lowest quintiles of total white meat, the following findings were observed:

- All-cause mortality: 25% decrease in risk [HR 0.75 95% CI 0.74-0.77]
- Cancer: 22% decrease in risk [HR 0.78 95% CI 0.75-0.81]
- Heart disease: 22% decrease in risk [HR 0.78 95% CI 0.75-0.82]
- Stroke: 13% decrease in risk [HR 0.87 95% CI 0.77-0.97]
- Diabetes: 31% decrease in risk [HR 0.69 95% CI 0.60-0.79]

Substitution analysis revealed similar magnitudes of risk reduction that observed with high intakes of white meat, with a minimum 20% reduction in risk for the disease outcomes outline above.

In the mediation analysis of meat constituents, the following findings were observed. Heme iron mediated 22.8% and 24.1% of the effect between processed meat and cancer and cardiovascular disease [CVD], respectively; nitrates mediated 37.0% and 72.0% of the effect for cancer and CVD, respectively, indicating a stronger effect of nitrates in processed meat than heme iron. For unprocessed red meat, heme iron exhibited a stronger mediating effect, statistically accounting for for 32.7% of the relationship with cancer, and 14.3% of the relationship with CVD.

*Geek Box: Confounders, Correlations, and Control

There is a tendency when it comes to interpreting epidemiological findings to dismiss any related factor between an exposure and and outcome as a "confounder". This ranges from over simplistic, to plain wrong. In order to be a confounder, a variable has to be associated with the exposure but not caused by it, and independently associated with the outcome. For example, an analysis looks at coffee as the exposure and heart disease as the outcome, and finds a strong association; but high coffee drinkers in the study are also heavy smokers. Coffee does not cause smoking, but they are related behaviours. In this case, controlling for smoking means the relationship between coffee and heart disease is not longer evident, i.e., smoking was the confounder. Many of the factors that we deem 'confounders' may in fact only be correlated behaviours or variables. The question to ask from a nutritional perspective is whether the dietary association is independent of non-dietary related lifestyle factors. We can determine this through appropriate control of known variables which may be correlated with diet, like socio-economic status, alcohol intake, or BMI. These variables are not inherently confounders; it depends on what the exposure-outcome relationship is that we're looking at. A common misconception reading such a list of covariates is to assume that all are confounders, however, this is incorrect; there are distinct differences between confounders [i.e., smoking], and moderating or mediating factors [i.e., fibre, fruit]. A general lack of understanding for the differences between such variables in widespread in discourse surrounding nutritional epidemiology. However, a fundamental difference is that a confounder may have direct relationship with the outcome, while a moderating factor may influence the size of the effect and the full operation of a cause-effect relationship, however, it does not invalidate that a relationship exists between the exposure and the outcome. If these were true confounders, then once they are adjusted for in the statistical analysis, the exposure-outcome relationship would no longer be evident. If the exposure-outcome relationship survives this adjustment, then it indicates that the effect of the exposure on the outcome is independent of these related non-dietary variables. While the caveat of epidemiology is always that "residual confounding cannot be ruled out", in reality it can't be ruled out in an RCT either, it's simply that randomisation is deemed to equally distribute unknown variables between an intervention and control group. The reality is that residual confounding implies there is something we don't know which could influence the results, which is always true; what is important to remember is that there is a lot we do know, and we can build that into an adjustment model to control for these variables. Remember: correlation does not imply confounding.

The Critical Breakdown

Pros: The study targeted recruitment areas with large minority populations. The cohort had enormous size, long follow-up period, and wide contrasts in dietary intake [more under Key Characteristic, below]. Over 128,000 deaths occurred during the follow-up period from all causes, which lent sufficient statistical power to investigate dietary constituents like heme, and nitrates/nitrites. Heme iron content of foods was quantified using advanced laboratory analysis of red meats. Three calibration studies were conducted to correct for measurement error in the dietary assessment method, with specific calibration for nitrates and nitrites, and for red meat intake. The confidence intervals are sufficiently narrow for the major findings to warrant both confidence in the direction of the effect, but also caution against minimising the findings.

Cons: For such a robust study in size, population, and calibration of the dietary assessment, it is a shame that the gold-standard validation of a 7-day measured food record was not undertaken to calibrate the initial FFQ. While the errors in a 24hr recall are independent of the errors in an FFQ, the caveat with 24hr recalls as the calibration instrument is they may result in biased estimates of the FFQ performance. However, this may not influence the results of the present study, given two more calibration studies were carried out for the specific exposures of interest.

Key Characteristic

The NIH-AARP Study was designed with the specific rationale of addressing two major methodological challenges of nutritional epidemiology [(4)]:

1) weak relative risks due to dietary measurement errors;

2) lack of sufficient contrast in exposure due to homogenous dietary intake in a region or population.

If an exposure is continuous, like diet, and if there is increasing measurement error [like dietary assessment], the requirement for sample size increases. The half-a-million participants in the NIH-AARP cohort increases the power to capture more valid measures of the exposure, and minimise the effects of measurement errors.

Secondly, such a large cohort recruited from diverse areas in the US provided a large variance in dietary intakes, and the contrast in exposure was sufficiently wide to yield meaningful comparisons, and therefore elucidate effects. For red meat, for example, the contrast ranged from the lowest median of 23.25g/d to 156.25g/d for a 2,500kcal/d diet.

Finally, numerous calibration studies have been undertaken to correct for measurement error [5,6]. In the present study, spectrometry [advanced lab technology] was used to quantify the heme iron content of specific foods, and validated the lab samples agains selected meat samples. This gave a quantitative level of heme iron to multiply against the amount of meat reported in the FFQ. A specific calibration study was used for nitrates and nitrites, which yielded reliable correlation coefficients comparable to many macronutrients. A final calibration analysis was undertaken to correct for errors in estimating red meat intake from the FFQ, in a subgroup of 1,877 participants.

The calibration studies in relation to nitrates/nitrates, and in relation to red meat intake, put this prospective study cohort above any others which have investigated red meat and health outcomes in terms of methodology. The size and contrast in exposure, coupled with the error-corrected estimates of red meat, heme iron, nitrates and nitrites, make the NIH-AARP perhaps the most robust study to investigate the relationship between meat and health outcomes.

Interesting Finding

The relative risk for all-cause mortality was higher in participants with higher intakes fruits and vegetables. Why could this be?

The calibration study for nitrates and nitrites revealed that average daily nitrate intake was 85mg/d in men and 91.6mg/d in women. 94–95% of total dietary nitrate intake on average was derived from plant sources [79mg/d and 86mg/d in men and women, respectively. The largest contributions to nitrate intake were from spinach, lettuce, and other greens. In the analysis, nitrates from processed meats mediated 50.1%, 37.0% and 72.0% of the relationship between processed red meat and all-cause, cancer and CVD mortality, respectively. This effect was stronger than the mediating effect of heme iron.

The authors do acknowledge that the greater risk in participants with higher vegetable and fruit intake may be due to biological interaction, as vegetables and fruits are rich sources of nitrates. While this may be true, the obverse of the coin is that we cannot just dismiss the findings in relation to meat and meat compounds as a reflection of a low vegetable and fruit intake, which is often a common excuse.

Thus, while it may be tempting to myopically focus on fibre in the context of colon cancer prevention, diet is always the sum of its part: the dietary fat composition of Western diets may be a significant factor itself.

Relevance

Last year, a group self-titled as the 'Nutritional Recommendations (NutriRECS) Consortium' published a series of meta-analyses upon which they based their own "new dietary guidelines" that recommended individual' s continue with current levels of meat consumption, on the basis of "low certainty" evidence for reducing meat intake benefiting health outcomes [7].

Closer scrutiny revealed the actual results were consistent with the wider literature, for example, a 24% reduction in risk for diabetes and 25% reduction in risk for stroke, comparing the highest to lowest levels of red meat intake. What the recommendations were based on was not the findings, but the certainty rating of the findings as assessed by an evidence evaluation tool [the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria] which is unsuited to evaluating nutrition science. The ultimately recommendations were incongruent with the actual evidence for the relationship between red meat and health outcomes.

Nutritional epidemiology faces two methodological challenges, as stated above: a lack of sufficient contrast in exposures [i.e., low variability in intake of an exposure of interest], and lack of sufficient numbers in the cohort to account for the degree of measurement error inherent in dietary assessment methods. The NIH-AARP cohort was designed to overcome these limitations, as have others, including the the European Prospective Investigation into Cancer and Nutrition [EPIC] study.

The reality is that every large, robustly conducted prospective cohort with sufficiently wide exposure contrasts and adequate statistical power in the sample size, has found strong relationships between high red meat intake and adverse health outcomes. Accounting variables like BMI, alcohol, smoking status, fruit and vegetable intake, baseline health status, and vitamin supplements, the main effects described above remained statistically significant in the NIH-AARP. In particular, the findings here corroborate mechanistic understanding of the roles of dietary constituents that are high in red meats, in particular heme iron, and processed meat nitrates and nitrites.

Science-based healthcare requires that we explain findings, not excuse them or diminish them because we don' t like what appears on paper.

Application to Practice

Risk in nutrition is a function of dose x duration of exposure. The data suggests that when it comes to red meat, keep the dose moderate, and the duration intermittent. A case can be made that for processed meat, the optimal dose is close to zero.

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