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ORIGINAL RESEARCH

Grilling the data: application of specification curve analysis to red meat and all-cause mortality

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Abstract

Objectives: To present an application of specification curve analysis—a novel analytic method that involves defining and implementing all plausible and valid analytic approaches for addressing a research question—to nutritional epidemiology.

Study Design and Setting: We reviewed all observational studies addressing the effect of red meat on all-cause mortality, sourced from a published systematic review, and documented variations in analytic methods (eg, choice of model, covariates, etc.). We enumerated all defensible combinations of analytic choices to produce a comprehensive list of all the ways in which the data may reasonably be analyzed. We applied specification curve analysis to data from National Health and Nutrition Examination Survey 2007 to 2014 to investigate the effect of unprocessed red meat on all-cause mortality. The specification curve analysis used a random sample of all reasonable analytic specifications we sourced from primary studies.

Results: Among 15 publications reporting on 24 cohorts included in the systematic review on red meat and all-cause mortality, we identified 70 unique analytic methods, each including different analytic models, covariates, and operationalizations of red meat (eg, continuous vs quantiles). We applied specification curve analysis to National Health and Nutrition Examination Survey, including 10,661 participants. Our specification curve analysis included 1208 unique analytic specifications, of which 435 (36.0%) yielded a hazard ratio equal to or more than 1 for the effect of red meat on all-cause mortality and 773 (64.0%) less than 1. The specification curve analysis yielded a median hazard ratio of 0.94 (interquartile range: 0.83−1.05). Forty-eight specifications (3.97%) were statistically significant, 40 of which indicated unprocessed red meat to reduce all-cause mortality and eight of which indicated red meat to increase mortality.

Conclusion: We show that the application of specification curve analysis to nutritional epidemiology is feasible and presents an innovative solution to analytic flexibility.

Keywords: Nutrition; Red meat; All-cause mortality; Multiverse analysis; Specification curve analysis; Vibration of effects

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Plain language summary

Randomized trials represent the optimal design for investigating the health effects of medical interventions. They pose important challenges, however, when it comes to studying the health effects of food and nutrition. Hence, investigators commonly perform nutritional epidemiology studies. These studies are observational in design, collect information from large groups of people, and look for patterns between their diet and their health.

There are, however, concerns about the trustworthiness of nutritional epidemiology studies, exemplified by cases where they have produced inconsistent results. A growing body of evidence suggests that these inconsistent findings may be explained by differences in analytic choices (ie, different ways of analyzing the same data). When investigators analyze nutritional epidemiology studies (and other types of observational data), there are often hundreds of equally justifiable ways of analyzing the data, each of which may produce different results. Hence, investigators may perform several analyses and selectively report results for the analysis, that is, most interesting or publishable.

In this study, we apply a novel analytic method—called specification curve analysis—to investigate the effect of red meat on all-cause mortality. This method involves defining and implementing all plausible and justifiable analytic approaches for addressing a research question. Investigators can subsequently consider the range of all plausible results and express more confidence in results that are consistent across all or most justifiable analytic specifications.

Our work suggests that specification curve analysis can be useful for studying the effects of diet on health. It provides a practical and new way to deal with the challenge of analytic flexibility. Broader application of specification curve analysis, along with other methods, may improve the credibility of such studies.

What is new?

Key findings

- The analysis of nutritional epidemiology data is complex and there is often limited consensus among experts about the ideal approach.
- While discrepancies in analytic models may result from differences in opinions regarding the optimal analytic approach among well-intentioned investigators, some investigators may test many alternative analytic specifications and selectively report results for the analysis that yields the most interesting findings.

What this adds to what was known?

- We apply a novel analytic method—called specification curve analysis—to investigate the effect of red meat on all-cause mortality. This method involves defining and implementing all plausible and justifiable analytic approaches for addressing a research question.

What is the implication and what should change now?

- We show variability in results across plausible analytic specifications.

- This research demonstrates how specification curve analysis can be effectively applied to nutritional epidemiology, providing a practical and innovative solution to the problem of analytic flexibility.

1. Background

Unlike randomized trials for which investigators typically register protocols and statistical analysis plans before the collection of any data, when investigators analyze data from observational studies, there are often hundreds of equally justifiable ways of analyzing the data, each of which may produce results that vary in direction, magnitude, and statistical significance [1—7]. The variability of effect estimates due to alternative analytic approaches is called ‘vibration of effects’ [2]. Empirical evidence shows that results from observational studies may be highly dependent on analytic choices [1—5].

While our empirical and theoretical understanding of the question being investigated should guide our analytic choices, our knowledge of complex biomedical and environmental systems is limited and even experienced investigators often come to different conclusions about the ideal analytic approach [4,6,8—13].

While we anticipate that discrepancies in analytic models often result from differences in opinions regarding the optimal analytic approach among well-intentioned investigators, some investigators may test many alternative analytic specifications and, intentionally or unintentionally, selectively report results for the specification that yields the most statistically significant or interesting results or results that support their preconceived hypotheses. Evidence shows that investigators' prior beliefs and expectations influence their results [5]. In the presence of strong opinions, investigators' beliefs and expectations may shape the literature to the detriment of empirical evidence [5].

Box 1 Specification curve analysis

When investigators analyze data from observational studies, they may make numerous potentially justifiable, but still subjective, analytic decisions on which the direction, magnitude, and statistical significance of results may be contingent. Specification curve analysis may mitigate this issue [26].

Specification curve analysis involves defining and implementing all plausible and justifiable analytic methods for investigating a research question. Investigators subsequently interpret the distribution of results across all plausible analyses, instead of focusing on the results of only one analysis.

The implementation of specification curve analysis involves:

(1) Defining all plausible choices across all aspects of the analysis. This typically includes:

  - Criteria for selecting eligible participants for inclusion in the analysis
  - Type of analytic model (eg, logistic, Poisson, or Cox proportional hazards models)
  - Choice of covariates
  - Operationalizations of the exposure variable and covariates (eg, transformations, functional form)

(2) Enumerating all justifiable combinations of these analytic choices to produce a comprehensive list of all the ways in which the data may be reasonably analyzed. For example, three unique choices for five aspects of the analysis yield 243 unique analytic specifications ($=3^5$).

(3) Implementing all or a random sample of all reasonable analytic specifications.

(4) Ordering the effect estimates from all analyses based on their direction and magnitude and presenting results on a specification curve plot. A specification curve plot reports the results of all analyses at the top and analytic characteristics at the bottom. The specification curve plot visually communicates the distribution of results across all specifications and the aspects of the analysis that are most consequential in influencing the direction and magnitude of findings.

1.1. Nutritional epidemiology

Nutrition is a field particularly amenable to analytic flexibility [14]. Trials investigating the health effects of nutritional exposures are often not feasible and so the evidence is primarily comprised of nutritional epidemiology studies—observational studies that recruit large groups of people and look for patterns between diet and health [15,16].

The analysis of nutritional epidemiology data is complex and there is often limited consensus among experts about the ideal approach [17,18]. Sources of analytic flexibility include the type of analytic model (eg, Poisson regression, Cox proportional hazards model), choice of covariates (ie, investigators studying the same question will consider different adjusting variables [19]), operationalization of the exposure variable and covariates in the model (eg, transformations, categorizations of continuous variables, functional form), and methods to address missing data, among others [8]. Investigators often present several sensitivity analyses to investigate the effects of these uncertain analytic decisions on the results, but the choice of sensitivity analyses is also subjective and investigators may be more inclined to report sensitivity analyses that affirm their primary findings.

A large body of evidence shows inconsistency in the results of nutritional studies, some of which may be explained by analytic flexibility [3,8,20,21]. Such inconsistencies have eroded trust in nutritional epidemiology and subjected the field to criticism [22,23]. Nevertheless, nutritional epidemiology studies continue to play a crucial role in shaping dietary recommendations and policies, making it imperative to draw credible inferences from these studies [14,15,24,25].

1.2. Specification curve analysis

Specification curve analysis—sometimes called multiverse analysis—is a novel analytic method that involves defining and implementing all plausible and valid analytic approaches for addressing a research question [26] (Box 1).

Through this approach, investigators define all plausible and justifiable choices for all aspects of the analysis (eg, choice of model, covariates, etc.), enumerate all justifiable combinations of these choices to produce a comprehensive list of all the ways in which the data may be reasonably analyzed (i.e., analytic specifications), implement all or a random sample of the valid analytic specifications, and draw inferences using the distribution of results from all plausible and justifiable specifications.

Specification curve analysis offers advantages to conventional methods for data analysis. It allows investigators to draw more credible inferences that are not contingent on arbitrary analytic decisions and reduces the opportunity for investigators to conduct many analyses and selectively report results for analyses that yield the most interesting results, although it does not completely eliminate subjectivity in analytic decisions.

While specification curve analysis has been previously applied in psychology and economics, it has seldom been applied in nutritional and environmental epidemiology [5,27].

1.3. Objectives

We apply specification curve analysis to investigate the effect of unprocessed red meat on all-cause mortality—a question that has yielded inconsistent results in the literature and produced conflicting dietary recommendations. While this study may provide insights on the health effects of red meat, the primary objective is to demonstrate the application of a novel analytic method—specification curve analysis—to nutritional epidemiology.

A critical limitation of specification curve analysis is the subjectivity involved in selecting justifiable analytic specifications. Investigators may disagree about justifiable analytic approaches or may present results of analyses that are only marginally justifiable. To mitigate this issue, our analytic specifications were informed by the most common analytic methods used in previous published studies addressing the effects of red meat on all-cause mortality.

2. Methods

This study was exempt from institutional ethics review because it uses secondary deidentified data. We report our results according to Strengthening the Reporting of Observational Studies in Epidemiology reporting guidelines for observational studies [28].

2.1. Analytic specifications

We used a published systematic review of observational studies that addressed the effect of red meat on all-cause mortality to identify justifiable analytic specifications for specification curve analysis [29]. We focus only on observational studies because randomized trials typically involve the preparation of detailed protocols and statistical analysis plans that reduce the analytic decisions available to investigators. While our objective was to investigate the effects of unprocessed red meat, we did not anticipate that studies investigating the effects of mixed unprocessed and processed red meat or unspecified types of red meat would use different analytic methods. Hence, we also reviewed studies that reported on mixed unprocessed and processed red meat and unspecified types of red meat.

Two reviewers, working independently and in duplicate, reviewed the primary studies from the systematic review and collected data on study characteristics and analytic methods, including the type of analytic model (eg, Cox proportional hazards model, logistic regression), method of adjustment for energy (eg, standard model, multivariable nutrient density model), covariates included in the model, operationalization of covariates (eg, categorical, linear, quadratic), subgroup analyses (eg, men vs women), and the results of analyses, including secondary and sensitivity analyses, when reported [29]. To ensure that the primary studies that we used to inform our analytic specifications addressed similar causal questions and interpreted their findings similarly, we documented the objectives of the primary studies and the ways in which the authors interpreted their findings.

2.2. Study population

The National Health and Nutrition Examination Survey (NHANES) is a repeated cross-sectional probability survey by the US Centers for Disease Control and Prevention to characterize the health and nutritional status of the noninstitutionalized, civilian US population [30]. The survey is based on household interviews and physical examinations and is representative of the US population by its survey sampling method. The survey collects demographic, socioeconomic, dietary, and health-related data by household interview, and medical, dental, physiological measurements, and laboratory tests by physical examination.

For this analysis, we used the continuous 2007–2014 NHANES data linked with the National Death Index [31] and the Food Patterns Equivalents Database. The National Death Index is a database established by the National Center for Health Statistics that contains information on all deaths in the United States. We extracted mortality status from the National Death Index up to December 31, 2019. The Food Patterns Equivalents Database contains

Box 2 Aspects of the analysis that varied across analytic specifications

(1) Type of nutrition model

- Standard model
- Multivariable nutrient density model

(2) Operationalization of red meat

- Continuous (per 100 g/day)
- Quartiles
- Quintiles

(3) Subgroups of interest

- All participants
- Subgroup based on sex
  - All females
  - All males
  - Both sexes
- Subgroups based on age
  - Participants aged 20–39 years
  - Participants aged 40–59 years
  - Participants aged 60–79 years
  - All ages

(4) Covariates

information on the composition and nutritional content of individual foods.

We acknowledge that NHANES data are likely suboptimal compared to other nutrition datasets for investigating the effect of red meat and other nutritional exposures on health outcomes, due to it including few deaths and only collecting data on diet at a single point in time [30,32]. Our objective, however, is not to provide answers about the health effects of red meat but to demonstrate a proof-of-concept application of specification curve analysis to nutritional epidemiology. We used NHANES data due to its availability to our team and our team's familiarity with its structure.

We observed that nearly all primary studies excluded participants with missing data and performed complete case analysis. We applied the same approach and excluded participants with missing demographic, dietary, or lifestyle information. Furthermore, we excluded pregnant people since they were not included in any of the primary studies. We also excluded participants with implausible body mass index (BMI) (<15 or ≥ 60 kg/m^2) or energy intake ($<$ 500 kcal/day or $>$4500 kcal/day) since these likely represent instances of inaccurate reporting or collection of data. To minimize missing data, we consolidated related variables in the database (eg, when data were missing for the smoking history variable, we classified participants who endorsed smoking 0 cigarettes in their life as nonsmokers).

Participants in NHANES completed two 24-hour dietary recalls, each conducted by trained interviewers and separated by 3−10 days, for which they provided information on intake of foods and beverages on each recall day [32]. For our analysis, we define unprocessed red meat as any mammalian meat (ie, beef, veal, pork, lamb, and game meat) [33].

2.3. Data analysis

We performed specification curve analysis to investigate the effects of unprocessed red meat on all-cause mortality, using a Cox proportional hazards regression model with time since 24-hour recalls as the time variable in the model.

For each aspect of the analysis, we used the most used analytic choices from previous studies (Box 2) and enumerated all combinations of these choices (within the context of the analytic choices that we had selected for consideration in the specification curve analysis) to produce a comprehensive list of all plausible and reasonable analytic methods. We reviewed analytic specifications to confirm that every combination of analytic choices implemented in the specification was indeed justifiable. Although we intended to exclude specifications comprised of combinations that were not defensible, we found no such cases.

Aspects of the analysis that varied across primary studies included the type of nutrition model (ie, standard model and multivariable nutrient density model), operationalization of red meat (ie, continuous, quartiles, quintiles), subgroups of interest (ie, only males, only females, all sexes, 20−39 years old, 40−59 years old, 60−79 years old, all ages), and choice of covariates. The standard nutrition model adjusts for total energy in the analytic model, while the multivariable nutrient density model divides food intake by total energy intake and also includes total energy intake in the model [34]. We did not consider the residual energy model since it is largely equivalent to the standard model [34].

We constructed two sets of covariates: covariates that we included in all models and covariates that were adjusted in some models. In all models, we adjusted for a core set of covariates that were considered in nearly all primary studies: age, sex, smoking, total energy intake, year, menopausal status, hormone therapy, parity, and oral contraceptives. We also optionally adjusted for a secondary set of other covariates that were only adjusted in some (but not all) studies: race/ethnicity (Mexican American/other Hispanic/non-Hispanic White/non-Hispanic Black/other race−including multiracial), education (less than 9th grade/9−11th grade/high school graduate/some college or AA degree/college graduate or above), marital status, alcohol consumption, physical activity, BMI, socioeconomic status, comorbidities, and dietary variables.

We are unable to test for all possible combinations of covariates due to computational feasibility. Hence, we generated 20 random unique combinations of covariates that all adjusted for the core set of variables and each of which adjusted for a random set of the secondary covariates. We applied specification curve analysis and computed hazard ratios (HRs) and 95% confidence intervals corresponding to the effect of red meat intake on all-cause mortality for each analytic specification.

For specifications in which red meat was treated as a continuous variable, we calculated HRs and associated confidence intervals corresponding to a 100 g/day increase in intake of red meat. For specifications in which red meat was treated as a categorical variable (eg, quartiles or quintiles), we calculated HRs and associated confidence intervals corresponding to the highest vs lowest quantile of red meat exposure. While these contrasts represent different quantities of red meat intake, primary observational nutritional epidemiology studies overlook these differences when interpreting results and systematic reviews and meta-analyses often combine these estimates from studies using disparate quantities [25]. In our supplement, we present results stratified by how red meat is defined in analytic models (ie, quartiles, quintiles, or continuous 100 g/day).

To test whether models from the specification curve analysis met the proportional hazards assumption, we selected a sample of all specifications at random and tested the correlation between Schoenfeld residuals and ranked failure time.

We excluded results from models that yielded what we considered to be implausible effect estimates (ie, studies that yielded implausibly wide confidence intervals with lower bound HR ≤ 0.2 or upper bound HR ≥ 5). A review of analytic specifications that yielded results outside of this range suggested sparse data bias, where there are too few events in certain combinations of explanatory variables resulting in overestimation or underestimation of effect estimates [35]. While these thresholds are arbitrary, they pragmatically excluded specifications that yielded what we considered to be results beyond the range of effects we would plausibly expect from diet and nutrition on health outcomes.

We performed three statistical tests to address (1) whether the median effect estimate across all specifications is more extreme than would be expected if red meat had no effect on all-cause mortality, (2) the proportion of specifications that produced statistically significant effects is more extreme than would be expected if red meat had no effect on all-cause mortality, and (3) whether Stouffer's averaged Z value across all specifications is more extreme than would be expected if red meat had no effect on all-cause mortality [26]. To perform these tests, we permuted red meat intake and sampled with replacement across all participants to yield 500 bootstrapped samples to which we applied specification curve analysis. Based on the results of the specification curve analysis to the permuted datasets, we calculated P values using the percentage of bootstrap sample with results as or more extreme than the observed results. We used an alpha of 0.05 to indicate statistical significance.

We performed all analyses in R (Vienna, Austria; version 4.1.2), using the *specr* package for specification curve analysis [36]. Data from NHANES are publicly accessible and the code to produce the results in this paper is available on a public repository: https://github.com/Yumin-Wang/Red-Meat-Consumption—All-Cause-Mortality.

3. Results

3.1. Study characteristics

A systematic review identified 15 publications reporting on 24 cohort studies that examined the effect of red meat on all-cause mortality [29] (Supplement Table 1).

To ensure that these primary studies addressed similar causal questions and interpreted their findings similarly, we documented the objectives of the primary studies and the ways in which the authors interpreted their findings (Supplement Table 2). The primary aim of all except two of these studies was to investigate the effects of red meat on all-cause mortality. One study investigated the effects of substituting total and different types of dietary protein for carbohydrates on mortality but also presented models investigating the effects of isocaloric substitutions of carbohydrates for red meat on mortality [37]. The second study investigated the effects of components of a traditional Sami diet, including red meat, on mortality [38].

Studies reported 70 unique methods to investigate the effect of red meat on all-cause mortality (Supplement Tables 2 and 3). Studies varied in their choice of analytic model (eg, Cox proportional hazards model, Poisson regression), adjustment for energy (eg, standard model, multivariable nutrient density model), covariates included in the model, operationalizations of variables (eg, functional form in the model), and subgroups. Typical studies performed time-dependent Cox regression models in which red meat was treated as a categorical variable in quartiles or quintiles and adjusted for age, sex, smoking, alcohol intake, physical activity, and BMI.

Studies reported relative effect estimates of red meat on all-cause mortality ranging between 0.63 and 2.31 (median:

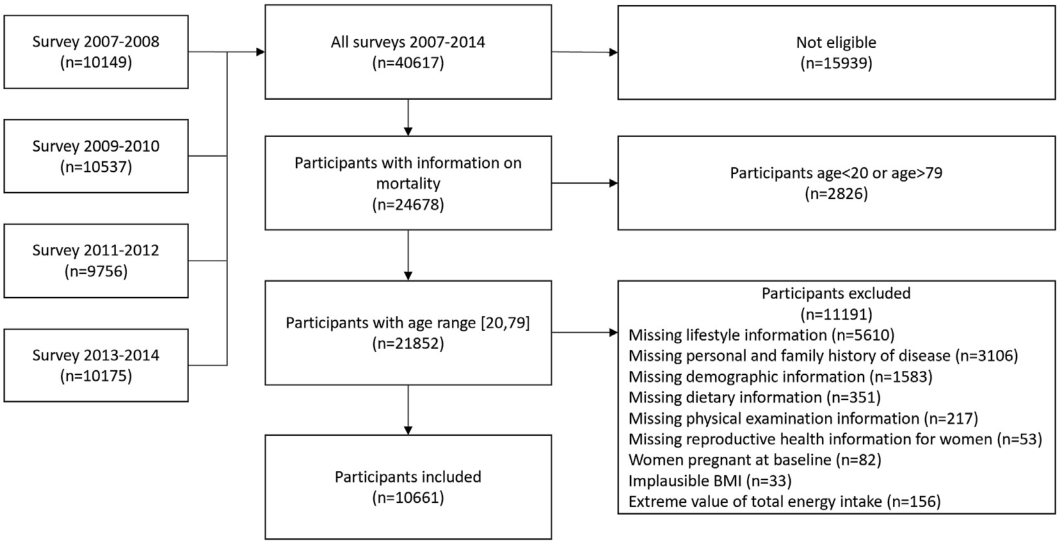

Fig. 1. Selection of study participants from the National Health and Nutrition Examination Survey (NHANES) for inclusion in the analysis.

1.14; interquartile range [IQR]: 1.02−1.23). Supplement Figure 1 presents the results of the analyses reported in studies.

3.2. Participant characteristics

We used data from NHANES 2007 to 2014 and excluded participants without mortality data and missing or implausible data, leaving 10,661 eligible participants. Fig. 1 presents the selection of participants in the analysis.

Table 1 and Supplement Table 5 present participant characteristics. Our study included participants ranging from young adults to the elderly, with approximately equal representation of men and women. Most participants were White, nonsmokers or light smokers, with a median intake of unprocessed red meat less than half a serving per day.

3.3. Specification curve analysis

Using all analytic choices identified in the primary studies, we enumerated all the ways in which the data may be reasonably analyzed (within the context of the analytic choices that we had selected for consideration in the specification curve analysis). The analytic methods varied according to the method of adjustment for energy (standard model, multivariable nutrient density model), the operationalization of red meat in the model (quintile, quartile, and continuous), subgroup based on sex (both sexes, male, female), subgroup based on age (all ages, 60−79 years old, 40−59 years old, 20−39 years old), and covariates. Each model was adjusted for a core set of mandatory variables and a random subset of 47 optional variables. Based on these variations in analytic choices, we calculated a total of 10 quadrillion possible unique analyses.

Table 1. Participant characteristics

Total participants, *N*	10,661
All-cause mortality, *n* (%)	1022 (10)
Follow-up (months)	99 (65, 143)
Age (years)	50 (27, 71)
Sex	
Female, *n* (%)	5150 (48)
Male, *n* (%)	5511 (52)
Dietary intakes	
Unprocessed red meat (g/d)	29.5 (0, 120.2)
Total energy intake (kcal/d)	1945 (1168, 3099)
Years of entering cohort	
2007−2008, *n* (%)	2311 (22)
2009−2010, *n* (%)	2358 (22)
2011−2012, *n* (%)	2857 (27)
2013−2014, *n* (%)	3135 (29)
Race/Ethnicity	
Mexican American, *n* (%)	1321 (12)
Other Hispanic, *n* (%)	988 (9)
Non-Hispanic White, *n* (%)	5193 (49)
Non-Hispanic Black, *n* (%)	2235 (21)
Other Race − Including Multiracial, *n* (%)	924 (9)
Smoking	
Nonsmoker or light smoker, *n* (%)	8373 (79)
Moderate smoker, *n* (%)	437 (4)
Heavy smoker, *n* (%)	1851 (17)
BMI (kg/m^2)	28.4 (21.9, 38.5)

Abbreviation: BMI, body mass index.

Data presented as numbers and proportions or as medians (10th percentile, 90th percentile).

Since we were unable to consider all possible unique analyses, we restricted the number of combinations of covariates we considered in the specification curve analysis. We generated 20 random unique combinations of covariates that all adjusted for the core set of variables and each of which adjusted for a random set of the secondary covariates. This yielded a total of 1440 unique analytic specifications. These 1440 analytic specifications represent a random subset of all 10 quadrillion possible analyses. We reviewed the 1440 specifications to confirm that every combination of analytic choices implemented in the specification curve analysis was indeed justifiable. Although we intended to exclude specifications comprised of combinations that were not defensible, we found no such cases.

We were able to accommodate most analytic choices reported in primary studies using data from NHANES (Supplement Tables 2 and 3). We were unable to implement time-varying variables due to the cross-sectional nature of the NHANES data.

We implemented 1440 reasonable specifications and identified 1208 unique specifications with plausible results and 232 with implausibly wide confidence intervals (lower bound HR $\leq$0.2 or upper bound HR $\geq$5). These implausible specifications occurred in analyses of subgroups of the total study population that included many adjusting covariates, suggesting sparse data bias [35].

Fig. 2 presents the results of the specification curve analysis. Our specification curve analysis produced a median HR of 0.94 (IQR: 0.83−1.05) for the effect of red meat on all-cause mortality. HRs ranged from 0.51 to 1.75. Of all specifications, 435 (36.0%) yielded HRs equal to or more than 1.0 and 773 (64.0%) less than 1.0.

Of all specifications, 48 (3.97%) were statistically significant. Of 48 statistically significant results, 40 had indicated red meat to reduce all-cause mortality and eight indicated red meat to increase all-cause mortality. Among statistically significant effects suggesting benefit, we observed a median HR of 0.65 (IQR: 0.58−0.69) and, among statistically significant effects suggesting harm, we observed a median HR of 1.22 (IQR: 1.19−1.27). We found 45% (542/1208) of all specifications to yield point estimates ranging between HR of 0.90 and 1.10.

Visual inspection of the specification curve plot suggests subgroup by sex to importantly influence results, with analyses restricted to women more likely to suggest red meat is beneficial. We observed a median HR of 1.05 (IQR: 0.89−1.12) for men and 0.85 (IQR: 0.77−0.93) for women. We did not identify other analytic characteristics as consequential.

Supplement Figure 2 presents the results of the specification curve analysis stratified by how red meat is defined in analytic models (ie, quartiles, quintiles, or continuous 100 g/day). Supplement Tables 6 to 10 and Supplement Figures 3 to 7 show the results of tests for the proportional hazards assumption and graphical displays of the correlation between Schoenfeld residuals and ranked failure time. We did not find evidence that the proportional hazards assumption was violated in any analyses.

Finally, we present statistical inferences about the degree to which findings across all specifications are inconsistent with the null hypothesis (ie, red meat has no effect on all-cause mortality) (Table 2). We performed statistical tests addressing whether (1) the median effect estimate across all specifications, (2) the proportion of specifications that produced statistically significant effects, and (3) Stouffer's averaged Z value across all specifications is more extreme than would be expected if red meat had no effect on all-cause mortality. All three statistical tests yielded P values $>.05$.

4. Discussion

4.1. Main findings

In this study, we applied specification curve analysis—a method that involves defining and implementing all plausible and valid analytic approaches for addressing a research question—to estimate the effect of unprocessed red meat on all-cause mortality [26]. To mitigate the subjectivity involved in selecting analytic specifications, we sourced analytic approaches from the literature [29]. We performed 1208 unique analyses and found considerable variability in results, with HRs ranging from 0.51 to 1.75. Our results suggest that findings in nutritional epidemiology studies may be contingent on analytic methods.

In contrast to previous studies addressing red meat, we found few of our analytic specifications to yield statistically significant effects. This may be because we used more recent data from NHANES, which include fewer accumulated deaths [39]. The most recent iterations of NHANES, however, are likely more reflective of the effects of red meat on all-cause mortality in the context of contemporaneous diets and lifestyles. Nevertheless, our primary objective was not to draw inferences about the health effects of red meat but to provide a proof-of-concept illustration of the application of specification curve analysis to nutritional epidemiology.

Concerns may arise over the impact of various analytic techniques on the interpretation of results. For example, different methods for energy adjustment may have different implications for how the effect is interpreted [18,40]. In our study, we show that despite differences in analytic methods, authors stated similar objectives and similarly interpreted their results. This suggests that authors are using disparate analytic methods to investigate near identical causal questions.

In addition to analytic flexibility, researchers criticize observational nutritional epidemiology studies for biases associated with self-reported dietary data [20,23]. Yet, nutritional epidemiology studies continue to play a critical

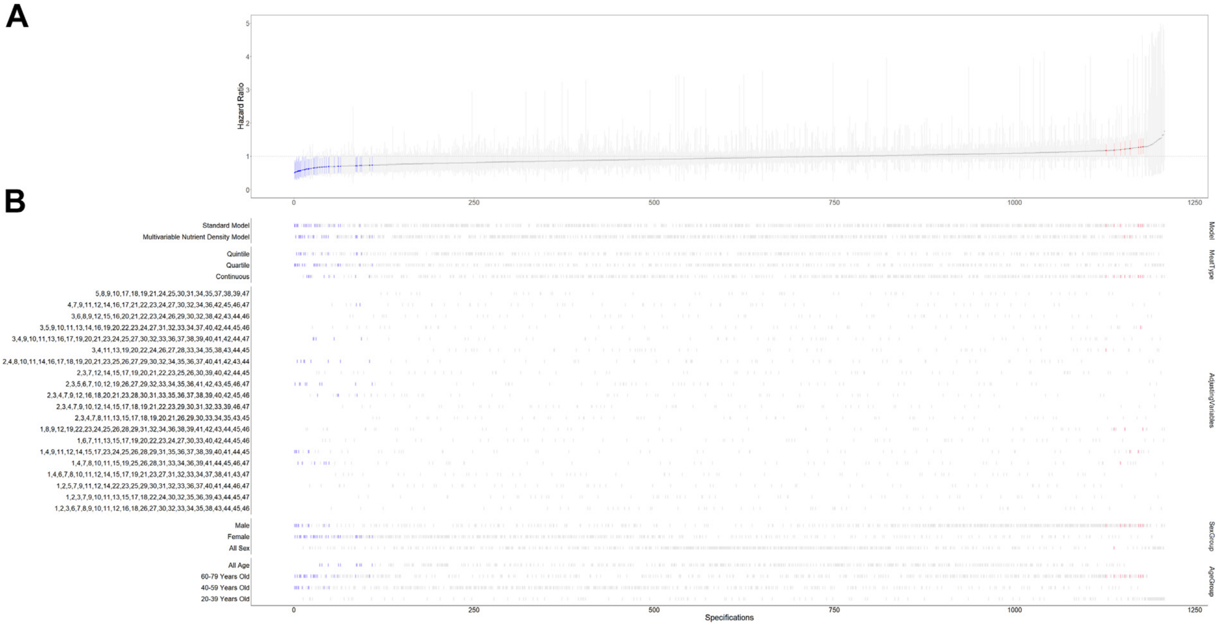

Fig. 2. Results of specification curve analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2. Inferential statistics

Test statistics used	Observed results	*P* value (% of bootstrap sample with results as or more extreme)
Median effect size	HR = 0.94	*P* = .472
Share of significant results	48 of 1208 specifications	*P* = .998
Aggregate all *P* values	Stouffer Z = −11.69	*P* = .732

Abbreviation: HR, hazard ratio.

role in shaping dietary recommendations and policies [15]. While specification curve analysis does not address biases due to dietary measures, when combined with other tools and methods for more reliably measuring diet, specification curve analysis may have the potential to enhance confidence in the discipline [41,42].

4.2. Relation to previous work

Current evidence shows that results from studies may vary due to alternative analytic specifications and that there is often limited consensus on the optimal approach for data analysis [6,43]. Research to date has not, however, quantified the magnitude of variation in results for typical epidemiologic questions. Furthermore, while specification curve analysis has been previously applied in psychology and economics, it has not yet been applied in epidemiology or nutritional epidemiology [27,44,45].

4.3. Strengths and limitations

The current work offers an innovative solution to analytic flexibility in nutritional epidemiology. To our knowledge, our work is the first application of specification curve analysis to nutritional epidemiology.

Our study also has limitations. There may be disagreements among investigators about what constitutes a justifiable analytic approach. To mitigate this issue, our choice of analytic specifications was informed by primary studies and so represents real, published analyses rather than possible, unpublished analyses that may only be marginally defensible. Ideally, investigators should prespecify criteria for distinguishing between justifiable and unjustifiable analytic approaches. We caution against investigators in making these distinctions after implementing the analysis since their decisions may be influenced by the observed results.

We emphasize that specification curve analysis does not eliminate subjectivity. For example, investigators may disagree about what constitutes a justifiable analytic approach. Furthermore, if investigators choose to select analytic specifications based on published literature, as we did in this study, there is typically more than one published systematic review that can be used to identify primary studies and the choice of systematic review may be subjective. Similarly, since there is usually more than one dataset available to address the same research question, the choice of dataset is also a subjective decision. As specification curve analysis becomes more common in epidemiology, we expect more of such subjective factors to emerge. Nonetheless, specification curve analysis does improve on current practice in which investigators can test many alternative analytic specifications and selectively report results for those that yield interesting or favorable results. It can identify findings that are most robust to alternative analytic specifications and encourage evidence users to interpret the results of epidemiology studies considering the typical variation in results expected due to analytic flexibility.

Specification curve analysis also does not eliminate the need for content knowledge and expertise. We see content expertise being essential to distinguishing between justifiable and nonjustifiable analytic specifications and interpreting and contextualizing results. In this study, content expertise in nutrition was critical to select methods to adjust for energy, the choice of core variables that we included in all analytic models, and the interpretation of our findings.

We did not register a protocol for the present study. This study is intended to provide a proof-of-concept rather than test any specific hypotheses. Since our work presents the first or one of the first applications of specification curve analysis to epidemiology, we expected to encounter many unanticipated decisions and challenges that we could not predict or describe in a protocol. Hence, our work was largely exploratory. The repository containing the analytic code also contains a history of the project from its inception in 2021.

Different analytic methods may have implications for the interpretation of results. For example, different methods to adjust for energy intake in nutritional epidemiology address different causal questions [18]. Authors of nutritional epidemiology studies, however, seldom acknowledge these issues. We show that despite differences in analytic methods, authors stated similar objectives and similarly interpreted their results.

We only applied specification curve analysis to one question—the effect of red meat on all-cause mortality. The extent to which results may be contingent on analytic methods may be different for other questions. We acknowledge that this is a controversial question in the nutrition literature and that the application of specification curve analysis to less contentious questions in nutritional epidemiology may improve its adoption. Our choice of topic was influenced by our team's familiarity with red meat and the related literature [15,29].

Our study likely underestimates the variations in results due to alternative analytic specifications since the analytic specifications that we could implement were limited by the availability of variables and data in NHANES. For example, due to the cross-sectional nature of NHANES, we were unable to use time-varying covariates and explore how alternative ways to account for these variables may influence results. We did not account for potential subjectivity in inclusion of participants in the analytic set (eg, thresholds for extreme energy intake) to maintain similar numbers of participants across analyses. Similarly, there are subjective analytic decisions in translating dietary recalls to nutrient and food intake, although we could not account for these decisions. For example, nutritional epidemiologists code dietary recalls according to food classification systems and subsequently use nutrition databases to estimate individual nutrient components of each item in dietary recalls—all of which involves subjective decisions.

The continuous 2007−2014 NHANES data are likely suboptimal for investigating the effect of red meat and other nutritional exposures on health outcomes, due to it including few deaths and only collecting data on diet at a single point in time [30,32]. Nevertheless, our primary objective is not to provide conclusive answers about the health effects of red meat but to demonstrate a proof-of-concept application of specification curve analysis to nutritional epidemiology.

We did not incorporate weights in our analytic models. Sample weights in NHANES are designed to account for oversampling of specific subgroups and unequal probabilities of selection in the population. These weights are essential when the objective is to make inferences about population characteristics or to estimate prevalence rates because they adjust for factors that influence these estimates and ensure that the results are representative of the target population. However, when focusing on causal inference, the primary concern is to eliminate or control for confounding factors that may distort the true relationship between exposure and outcome and sample weights are less important, especially when variables used to derive sample weights are already included in analytic models [46−48].

We excluded models that yielded results that we deemed to be implausible based on pragmatic but arbitrary thresholds (ie, HR ≤ 0.2 or HR ≥ 5). We suspect that the observed implausible specifications were due to sparse data bias—where there are too few events in critical combinations of explanatory variables [35]. It is, however, possible that there were other models that produced results within this threshold that had too few events to reliably estimate the effect of red meat on all-cause mortality.

Finally, while we attempted to test the proportional hazards assumption using the correlation between Schoenfeld residuals and ranked failure time, these tests have limited sensitivity [49]. We also only tested a proportion of our models for the proportional hazards assumption and it is possible that this assumption may be violated in models that we did not test.

4.4. Implications

Specification curve analysis allows investigators to test all plausible and justifiable models to explain conflicting findings or contextualize emerging findings. While this study may provide insights on the health effects of unprocessed red meat, we believe the most important contribution of this study is to provide a proof-of-concept demonstrating the feasibility of applying specification curve analysis to nutritional epidemiology.

Nutritional epidemiology has long been criticized for producing sensational and conflicting findings, which has eroded confidence in the discipline [23]. Nevertheless, nutritional epidemiology studies continue to play a crucial role in shaping dietary recommendations and policies, making it imperative to draw credible inferences from these studies [14,15,24]. The broader application of specification curve analysis to nutritional epidemiology may enhance confidence in nutrition as a field by encouraging investigators to acknowledge an additional source of uncertainty in studies. When combined with other tools and methods that also address other limitations of observational nutritional epidemiology studies (eg, biases that affect self-reported dietary data) [41], specification curve analysis has the potential to address a critical issue in epidemiology—analytic flexibility—and identify findings that are most robust to subjective analytic choices.

Findings from our study and future application of specification curve analysis will also be useful to evidence users who can interpret results of epidemiology studies in the context of the typical variation expected due to analytic flexibility. When effect estimates exceed the typical variation due to analytic methods, evidence users can be more certain of the findings, since they are likely robust to alternative analytic decisions.

Our findings may also have implications for precision nutrition that attempts to distinguish between subgroups of individuals who may differently respond to nutritional interventions or have different nutritional needs [50−52]. Investigators have raised concerns that efforts to identify ''responders'' and realize precision nutrition may be highly dependent on the characteristics of analytic models [53]. Specification curve analysis may be useful for evaluating the reliability of precision nutrition claims across a range of defensible models.

We acknowledge that the application of specification curve analysis is time-consuming and resource-intensive. Sourcing justifiable analytic specifications from primary studies adds to this effort. While the application of specification curve analysis may not be feasible for all nutritional epidemiology questions, it can be applied to the most critical, impactful, or contentious questions in the discipline and can serve as an additional available tool to evaluate the credibility of nutrition claims in the literature.

This is one of the first applications of specification curve analysis to epidemiologic health data. We anticipate further refinement of the method with future applications, including the development of more comprehensive guidance for investigators and increased standardization of the approach. For example, ideally, investigators should prespecify how they will select analytic aspects to consider in specification curve analyses and how they will distinguish between justifiable and unjustifiable analytic approaches. Furthermore, the interpretation of results from specification curve analysis is currently complex. Specification curve plots may be overwhelming for evidence users, especially if they account for many different analytic aspects. We hope with the greater adoption of this method, improved ways of communicating results from specification curve analyses emerge.

5. Conclusion

In this study, we apply specification curve analysis—a novel analytic method that involves defining and implementing all plausible and valid analytic approaches for addressing a research question—to investigate the effect of red meat on all-cause mortality. We show variability in results across plausible analytic specifications. This research demonstrates how specification curve analysis can be effectively applied to nutritional epidemiology, providing a practical and innovative solution to analytic flexibility. This approach has the potential to improve the credibility of inferences from such epidemiologic studies.

This figure presents the results of the specification curve analysis, including 1208 unique analytic specifications. The upper portion of the plot shows HRs representing the effect of red meat on all-cause mortality. On the x-axis are the unique analytic specifications. The y-axis represents the magnitude of effect estimates. Each point on the graph represents the results of a unique analytic specification. Point estimates are shown in dark gray and 95% confidence intervals as light gray bars. Each point represents the results for the effect of red meat on all-cause mortality for a unique model. Points in blue are statistically significant and suggest red meat to prevent all-cause mortality and points in red are statistically significant and indicate red meat to increase risk of all-cause mortality.

The lower part of the plot shows the characteristics of each analysis, including type of analytic model, operationalizations of variables, choice of covariates, and subgroups of interest. Each vertical line denotes the specific choice applied for each aspect of the analysis. We assigned a unique number to each covariate (Supplement 4 shows the number corresponding to each variable). Combinations of numbers in the graph represent combinations of covariates included in the model.

Ethics approval

Not required.

Patient/public engagement

It was not possible to involve patients or the public in the design, conduct, reporting, or dissemination plans of our research.

CRediT authorship contribution statement

Yumin Wang: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation, Conceptualization. **Tyler Pitre:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Joshua D. Wallach:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Russell J. de Souza:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Tanvir Jassal:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation. **Dennis Bier:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Chirag J. Patel:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis. **Dena Zeraatkar:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclinepi.2024.111278.

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