

Review

Publishing Nutrition Research: A Review of Epidemiologic Methods

BARBARA BRUEMMER, PhD, RD; JEFFREY HARRIS, DrPH, RD; PHIL GLEASON, PhD; CAROL J. BOUSHEY, PhD, MPH, RD; PATRICIA M. SHEEAN, PhD, RD; SUJATA ARCHER, PhD, RD; LINDA VAN HORN, PhD, RD

ABSTRACT

The use of epidemiologic research designs and analytical methods is common in dietetics research. Food and nutrition professionals who seek to perform evidence-based practice or participate in research design, analysis, and communication need skills in the essentials of epidemiology. This is one of a series of monographs on research methodology that addresses these needs and supports the goals of the Board of Editors of the *Journal of the American Dietetic Association* to further enhance competency and skills. This monograph focuses on statistical approaches for univariate analyses used with the primary observational study designs associated with epidemiology. Tables illustrating the presentation and interpretation of these results are included.

J Am Diet Assoc. 2009;109:1728-1737.

This monograph continues the series on research design and analysis (1-4) and reviews epidemiologic study designs that are well-suited for analysis of common questions encountered in nutritional science. Epidemiology has been defined by Gordis (5) as “the study

B. Bruemmer is a senior lecturer, Graduate Program in Nutritional Sciences, and director, Graduate Coordinated Program in Dietetics, University of Washington, Seattle. J. Harris is an associate professor and didactic program director, Department of Health, West Chester University of Pennsylvania, West Chester, PA. P. Gleason is a senior researcher, Mathematica Policy Institute, Inc, Geneva, NY. C. J. Boushey is an associate professor, and director, Coordinated Program in Dietetics, Purdue University, West Lafayette, IN. P. M. Sheean is an instructor, Department of Preventive Medicine, Linda Van Horn is a professor, Department of Preventive Medicine, and associate dean for Faculty Development, and S. Archer is research editor, Journal of the American Dietetic Association, Northwestern University Feinberg School of Medicine, Chicago, IL.

Address correspondence to: Barbara Bruemmer, PhD, RD, Graduate Program in Nutritional Sciences and Graduate Coordinated Program in Dietetics, University of Washington, 305 Raitt Hall, Box 353410, Seattle, WA 98195. E-mail: bbruemme@u.washington.edu

Manuscript accepted: April 6, 2009.

Copyright © 2009 by the American Dietetic Association.

0002-8223/09/10910-0006\$36.00/0

doi: 10.1016/j.jada.2009.07.011

of the distribution and determinants of health-related states or events in specified populations and the application of this study to control health problems.” Epidemiologic methods have been refined and used in genetic epidemiology (6), social epidemiology (7), field epidemiology (8), as well as specific issues for nutritional epidemiology (9). The target audience for this monograph is investigators who wish to strengthen skills or require review of the evaluation of evidenced-based practice. The business of research is dynamic, competitive, and costly. Efficiency of study resources should be targeted to the most powerful tools and current techniques. Only by ensuring the use of appropriate tools will the broader goal of health improvement be realized. Epidemiologic methods can support a wide range of research situations from descriptive studies to randomized controlled trials. Three key elements involved in epidemiologic research are common terms (Figure 1), a conceptual framework (Figure 2), and concepts of causal association. The elements of causal associations set forth by Hill (10) have assisted scientists in making inferences about causal links (Figure 3). Rothman (11) has further contributed to our understanding of these elements noting that the original tenets included “specificity,” which states that one cause leads only to a single effect; a concept which clearly has evolved.

EPIDEMIOLOGIC STUDY DESIGNS

An investigator begins the research process with development of the hypothesis that includes a conceptual causal factor and an outcome of interest. Once that has been decided the appropriate study design is selected (1). A cross-sectional study does not allow examining a sequence of events but rather examines associations only at one point in time. Advantages of this approach include that it is typically less expensive and study participants are generally able to respond about current habits or status. However a major limitation is that, as in the example provided below, a cross-sectional design cannot provide insight into a temporal sequence leading to evidence on a causal factor. In other words, cross-sectional studies cannot provide answers to cause and effect.

Conversely, a second common way to pose a research question is to focus on a known outcome or endpoint, such as cardiovascular disease, nutrient deficiency, or breast cancer, and to explore the antecedents that may have contributed to the outcome. When an investigator is looking for an exposure to a known outcome, a case-control study may be appropriate (12,13). This type of retrospective study may be efficient in that cases and controls usually can be easily identified in a sample from their outcome status. Then questionnaires can be used to ob-

| Term | Definition |
|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Exposure (21) (potential risk factor, independent variable, predictor of disease, or other health event) | A factor that is an antecedent to an outcome. An exposure may be a trait, characteristic, behavior, or other factor that is being investigated as a potential cause (21,30). In an epidemiologic study, those with the exposure are termed the study group; the unexposed are the controls. |
| Outcome (21) (effect, endpoint, dependent variable) | An event of interest that may be influenced by the exposure. Those with the outcome are compared to those without the outcome. |
| Cross-sectional study design | “A group examined at one point of time.” Thus the measure of the exposure of interest and the outcome of interest are measured at the same time and temporal sequence cannot be established. |
| Case-control study design | Two groups, defined according to the presence or absence of the outcome. The previous exposure of those with the outcome (cases) are compared to the previous exposure of those without the outcome (controls) to identify factors that may have influenced the development of the outcome. |
| Cohort study design | A group free of the outcome of interest that is followed over time. Start with exposure and follow forward to determine if members of the group end up experiencing the outcome. A cohort study may be retrospective or historical when the investigator establishes the baseline disease-free state at some time in the past and follows forward to document new disease. Or a cohort study may be prospective when the baseline period is in the current time frame and documentation of new disease will take place in the future. |
| Incidence vs prevalence | Cumulative incidence refers to a first event of an outcome is the number of new cases within a defined period of time. Prevalence is the total number of subjects with the outcome at one point in time. |
| Confounder | A factor that is related to both the exposure and outcome that may distort the relationship. |
| Reference group | When examining an exposure that is considered a potential risk factor, the group that is normally considered to have the lowest level of risk is designated the reference group. All other groups will be compared to this reference group. Note when examining a factor that may be protective the investigator may wish to compare all groups to a reference that is considered to have the highest risk. Ideally the reference group will be large enough to provide precision in the estimates and should be fairly homogeneous (31). |

Figure 1. Terms used in epidemiologic research and their definitions. NOTE: Information from this figure is available online at www.adajournal.org as part of a PowerPoint presentation.

tain information on subject characteristics and factors that may have influenced the etiology of the outcome at some specified time in the past. Food frequency questionnaires have often been used to assess diet in case-control studies where investigators are seeking information on past behaviors. A unique challenge in this study design is to appropriately target the time period in the past that represents the plausible biologic time period of exposure. In cancer studies for example, investigators must know if the hypothesized exposure is relevant in an early phase of cancer development (eg, initiation, or at a later phase, promotion). The study participant must be instructed to focus on a time frame for the usual intake relative to that period, such as 5 years before the interview or between 20 and 25 years of age. Clearly this is a cognitive challenge for individuals. It may also open the study to the phenomenon of recall bias where cases are motivated to search their memories in more detail because they are dealing with the psychological burden of disease. Because controls do not have the same motivation there may be bias in the responses from the two study groups. These tools were considered acceptable due to their relatively lower cost and previous reports of relative validity compared to food records. However, studies have recently documented that there are many limitations in assessing

prior diet and the validity of food frequency questionnaires has recently been reevaluated using more objective biomarkers for comparison (14-17). These issues will be further explored in a future monograph.

Questions that focus on an exposure or an antecedent to an outcome are explored in cohort studies that may be prospective studies or retrospective (1). The exposure may be any measureable event or physiological condition, such as weight gain, diet quality, or lack of policies that support breastfeeding, which may all lead to specific subsequent outcomes. For example, research questions such as: What are the consequences of weight gain?, Is a diet high in dark green leafy vegetables protective against hypertension?, and How long will a working woman breastfeed if she is not provided with a suitable place to pump breast milk? all apply to this situation. Each of these questions has identified a factor that can be temporally followed for an association with specified outcomes. The main advantage of the prospective cohort study is that multiple outcomes may be assessed over time. For example, many of the large national cohorts such as the Framingham Heart Study (17), the Nurses' Health Study (18), and others have gathered baseline data that may be used to evaluate not only the initial hypothesized outcome but other health events or emerg-

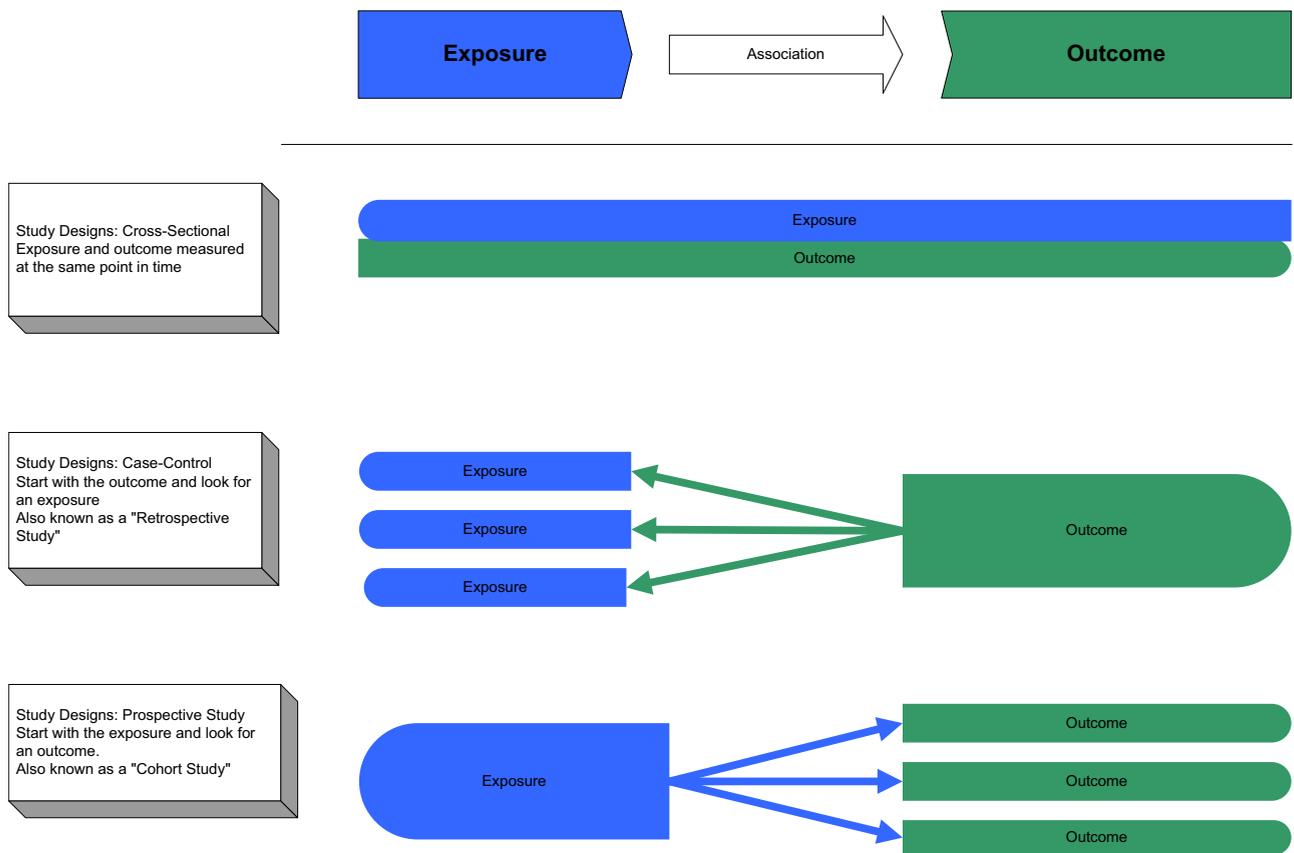


Figure 2. Schematic of epidemiologic study designs. NOTE: Information from this figure is available online at www.adajournal.org as part of a PowerPoint presentation.

| Criteria | Rationale |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Strength of Association | Strong associations are more likely to be causal than weak associations. |
| 2. Consistency | A causal association should be consistent across various study designs. |
| 3. Specificity | The rationale is quite controversial in that this tenet presumes that one cause is responsible for one effect, but we see clear evidence of redundancy in many biological systems. |
| 4. Temporality | The theoretical cause must precede the effect. |
| 5. Biologic gradient | The ability to demonstrate a dose-response relationship. Note this quality may not always be present. |
| 6. Plausibility | Is there a scientific justification for the relationship between cause and effect? Clearly this is a constantly evolving issue, such as the current evolving science in nutritional genomics where the consequences of for example genetic polymorphisms is not be known. |
| 7. Coherence | The central tenants of the causal association fit with other known characteristics of the cause and the effect. |
| 8. Experimental evidence | The existence of randomized controlled trials. (Unfortunately there is a paucity of randomized controlled trials in the field of nutrition.) |
| 9. Analogy | This element may be more the art of research than pure science. It refers to insight into the causal pathways and is considered a more controversial criterion because scientists are generally individuals of great creativity. |

Figure 3. Hill's (10) criteria for causal inference, as presented by Rothman (11).

ing health issues. The disadvantages are generally related to the cost of maintaining contact with a large number of study participants, the inefficiency of gathering information on individuals who may not contribute information on a rare outcome, and the limitations of our current diet assessment methods.

A variant of a cohort study is a nested case-control study that limits subject selection to members of a pre-existing cohort. This is a very efficient design because exposure information, such as biological samples may be gathered at baseline but may only be evaluated for those subjects who are later selected to participate in the case-control study based on disease or disease-free status. This is generally only a small subset of the larger cohort so the cost of analysis of the exposure information is reduced. An example would be a large cohort study where blood samples were collected at baseline and subjects were followed forward for 10 years. Subjects who developed cancer during the 10 years could be compared to a group of disease-free controls sampled from the cohort. A comparison of baseline blood samples between these cases and the controls would be much more efficient than analyzing the blood samples of all members of the cohort.

Issues in Study Validity

The methods or design used in any study may influence the internal validity or external validity of the study. The internal validity of a study means that the study was able to address the stated hypothesis because the methods were free of bias. The outcome must be evaluated without consideration of the exposure classification which would compromise the internal validity by “stacking the deck” and making it less likely that the analysis of the data will provide insight into the true relationship of interest. Factors that may introduce bias include selection bias or differential recruitment of study participants into the case vs control groups. Nonresponse bias may occur if survey responders and nonresponders differ systematically and the rate of nonresponse differs for those who have the disease/condition being analyzed vs those who do not. Recall bias, as noted previously in regard to food frequency questionnaires, compromises internal validity. Bias due to misclassification occurs when exposure information has been measured or recorded with error.

It is important to distinguish between nondifferential misclassification, which indicates the error in exposure measurement occurs with the same pattern among those with and without the outcome, and differential misclassification where the error in exposure measurement differs between those with and without the outcome. Nondifferential misclassification happens often in nutrition research when subjects are unable to accurately report usual dietary intake. If both the cases and the controls similarly misjudge their intake the result is a diminished ability to find an association when one exists or an attenuation of the results. Differential misclassification is more serious because the bias may actually contribute to a false association. For example, in a study on total energy intake and blood glucose, if a site with a high proportion of subjects with the metabolic syndrome has a higher rate of error in dietary records than a site with a low proportion of subjects with the metabolic syndrome, the pattern of error may contribute to a finding of an

association where one does not exist. This type of bias could even alter the direction of the finding suggesting a false association is protective or could be a risk factor.

The presence of one or more confounding factors is also a threat to internal validity. *Confounding factors, confounders, covariates, or adjustment variables* are all terms that refer to extraneous factors that may influence the association between the exposure and the outcome. If confounders are not identified or appropriately controlled for, the conclusions of the study may be flawed. A false association may be suggested between the exposure and the outcome that was due to the presence of the confounder. For example, if an investigator is interested in the association between body weight and mortality it would be important to identify subjects with cancer because this potential confounding factor is related to both body weight and mortality. Confounders may be identified through a number of methods such as a review of the literature, a review of pilot data, or a theoretical framework or conceptual model. Additional information on the potential for biased estimates due to inappropriate methods in the selection of confounding factors is provided by Harrell (19). In particular, the investigator will want to identify factors that are strongly associated with the outcome of interest. Another criterion for selection would be a factor that is associated with both the exposure and the outcome though the estimated strength may not be strong for either alone. The initial data analysis will provide the investigator with an opportunity to examine the relationship between the exposure and outcome with and without the confounder present. However, at the design stage, it is important to be inclusive and gather as much information on confounders as feasible. Typical confounders may be age, sex, education, income, and health behaviors such as tobacco use and exercise (1).

External validity refers to the extent to which the results from the study can be accurately generalized to the larger target population of interest. External validity may be compromised when study participants are not recruited from a representative sample of at-risk individuals. If the study participants are not representative of the target population the results will not be generalizable to that population. Data on response rates to questionnaires, participation rates, and loss to follow-up must be carefully gathered and analyzed. Many of the methods of analysis that are used in epidemiology may not be valid when studies are flawed by high rates of attrition or low response rates. The methods section should provide adequate information on how the exposure, outcome, and confounders were identified; how the information was captured; and how variables were categorized.

Preparation for Data Analysis

Beyond establishing the foundation for the research by stating a hypothesis, selecting the appropriate study design, and adhering to guidelines on methodology, the quality of an epidemiologic study also depends on having a well-considered plan for systematically gathering the data and subsequently effectively managing this information. Planning for analysis early in the execution of the project will assure a smooth transition from data gathering, through editing and into analysis and specific to randomized controlled trials is considered essential to

| Association | Code | Interpretation |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Positive association | | |
| Outcome in two categories | Outcome code | The coding and statistical analysis has been designed to reflect the hypothesis of a higher risk of mortality with a higher BMI ^a . If the finding has an OR ^b of 1.30 the summary would state the analysis suggests an increased mortality of 30% among those with a BMI \geq 30 compared to those with a BMI 18.5 to 24.9. (Note: this is an example where a summary statement for the lowest category should include the values. If the statement was “compared to the lowest BMI” that might be misinterpreted because BMIs of <18.5 were excluded from the study.) |
| Survival | outcome=0 | |
| Mortality | outcome=1 | Statistical programs may default the reference group to the lowest coded category unless otherwise stated. Therefore to model this hypothesis, code the better outcome (survival) as the lower number (zero) and the theoretical best exposure group (18.5 to 24.9) as the lowest category. Refer to the documentation for the statistical program for more information on defaults. |
| Exposure: BMI in three categories: | Exposure code | |
| \geq 30.0 | BMI=3 | |
| 25.0-29.9 | BMI=2 | |
| 18.5-24.9 | BMI=1 | |
| In this model, an RR ^c or OR >1 indicates a positive association; that is, the risk of death was higher in the BMI category \geq 30 compared to the reference group of 18.5 to 24.9. | | |
| Negative association | | |
| Outcome: | Outcome code | This coding and statistical analysis has been designed to reflect the hypothesis of protection with increased fruit intake. If the finding has an OR of 0.80 the summary would state the analysis suggests a decrease in mortality of 20% with fruit intake >3 servings/d compared to <1 serving/d. |
| Survival | outcome=0 | |
| Mortality | outcome=1 | For coding – note as above where the theoretical protective exposure category is coded as the highest number. Refer to the documentation for the statistical program for more information on defaults. |
| Exposure: Fruit intake in quartiles | Exposure code | |
| Quartile 1=<1/d | 1 | |
| Quartile 2=1-1.9/d | 2 | |
| Quartile 3=2-2.9/d | 3 | |
| Quartile 4=>3/d | 4 | |
| In this model, a RR or OR <1 for the highest quartile compared to the reference group for mortality indicates a negative association. As the intake of servings of fruit increases the risk of mortality decreases. This association may be described as a protective effect. | | |

Figure 4. Interpreting the direction of an association. These examples illustrate overall principles of epidemiologic research. ^aBMI=body mass index. ^bOR=odds ratio. ^cRR=relative risk.

drawing valid conclusions. Even simple decisions such as the use of a relational database in place of a spreadsheet may provide flexibility that speeds the data editing phase but also provides an increased level of security. A well-planned study design and implementation should also minimize missing data that can lead to bias.

Collecting as much raw data as possible will allow for flexibility in establishing categories during analysis. For example, if age is initially recorded as a continuous variable, it can be categorized as a dichotomous variable, such as <30 compared to \geq 30 years of age, or in tertiles, quartiles, or other proportions during the analysis phase. Often dichotomous distributions provide limited insight into the trend across categories and therefore may not be suitable (13). Proportional distributions, such as percentiles, may be determined based on all subjects, or based on only the controls or only the cases. Other categories appropriate for age are biological ranges such as premenarche, adult, postmenopausal; or chronologic age such as decades, age 20 to 29 years, or age 30 to 39 years. For body mass index (BMI) common approaches may capture the Centers for Disease Control and Prevention guidelines of 18.5 to 24.9 as desirable; 25 to 29.9 as overweight, and \geq 30 as obese (20). The

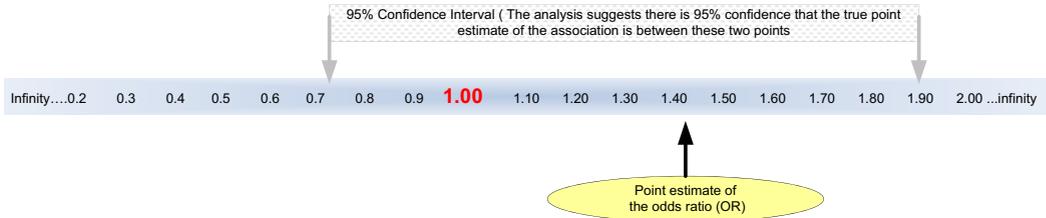
investigator may determine that individuals below a certain BMI may not be appropriate as a reference group. The decision to drop this group or to establish a reference group in the mid-range of BMIs should be based on the hypothesis of interest and scientific evidence. The number of subjects in each category level may differ between the cases and the controls but highly uneven categories may limit the statistical power of the analysis.

STATISTICAL ANALYSIS

The following is a brief description of the main univariate statistical tests that are used in epidemiologic studies. Multivariate methods will be described in a future monograph or the reader may wish to consult advanced texts (21-23). Each of these methods is a variation on a summary statement of the relationship between the exposure and the outcome. Outcome measures in epidemiologic studies are often presented as binary variables (ie, can potentially take on only two values) (24). This monograph presents the concepts of relative risks (RR) and odds ratios (OR) using binary examples. In particular, these measures tell the reader whether:

**Conceptual Model
No association**

Odds ratio = 1.40 (95% confidence interval: -0.72, 1.92) = Note the 95% CI includes 1.0 therefore the analysis suggests there is no association. The point estimate of the odds ratio may be due to chance. It could also be stated that the association is not significant at the $\alpha = 0.05$ level.



**Conceptual Model
Of an association**

Odds ratio = 2.4 (95% confidence interval: 1.2, 4.1) = Note the 95% CI does not include 1.0 therefore the analysis suggests we should not reject the null hypothesis. The point estimate of the odds ratio is unlikely to be due to chance. It could also be stated that the association is statistically significant at the $\alpha = 0.05$ level.

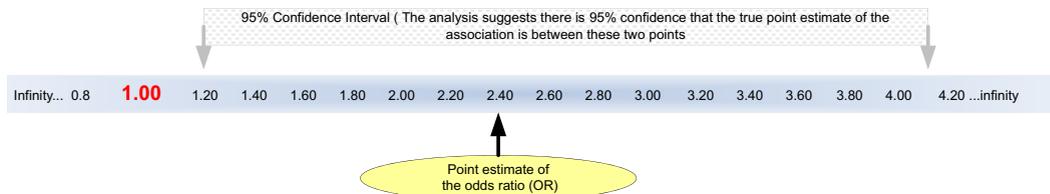


Figure 5. Conceptual models of odds ratios and confidence intervals (CIs). The models illustrate shifts in the placement and width of confidence intervals in associations with and without statistical significance. Note the inclusion of unity (=1) within the CI indicates that the association may be due to chance, which is the same as not statistically significant. Where one is not included in the CI the conclusion would be that the association is unlikely to be due to chance, which is the same as statistically significant.

- The exposure is positively associated with the likelihood of having the outcome (a RR or an OR >1),
- The exposure is negatively associated with the likelihood of having an outcome (a RR or an OR <1), and
- How much of a change relative to unity (where unity =1) was found.

For example, a point estimate of 1.4 strictly indicates that the risk of developing the outcome among those in the exposed group was 1.4 times the risk for those in the unexposed group. A summary statement may read, “There was a positive association between the exposed group compared to the unexposed group on the outcome of interest (OR 1.4).”

An investigator should pose the hypothesis with the potential direction of the association clearly identified. Then in the data gathering stage the information should be coded to mirror the question (Figure 4). A new investigator may lack an appreciation of how easily the results may be misinterpreted if the coding is not considered (25). When the results of a study are in conflict with the a priori hypothesis and/or the evidence in the literature, the first step should be a reconfirmation that the results are being interpreted correctly.

RR

The RR estimate is used in prospective studies. It represents the magnitude of an association between an expo-

sure and the likelihood of developing an outcome in the exposed group relative to the unexposed group (22). RR estimates are used in prospective studies under two options. The first option is a study that has counts of subjects according to their exposure and outcome status. The count of the outcome is generally for incident cases meaning the first time event during the follow-up period. The second option is a study that uses person time in the denominator. It is an estimate of the incidence of the outcome in the exposed group divided by that group’s cumulative time of the study (eg, in person-days) compared to the incidence in the unexposed group during that group’s cumulative time in the study. The RR as described above would be a crude estimate or univariate analysis because no confounders have been considered in the analysis. Prospective studies often employ more sophisticated models that allow for adjustment of confounders and inclusion of survival information (23,26) and graphic illustrations such as the Kaplan-Meier method. (27).

OR

A variant of the RR approach is used for cross-sectional and case-control studies where person time under a theoretical exposure period is not available. The OR is the odds of having the exposure among those with the outcome (cases) divided by the odds of having the exposure among those without the outcome. The OR is considered

Hypothetical scenario 1: *The association between the presence of a high fructose corn syrup beverage (HFCSB) in a household and the prevalence of obesity.*

Exposure

- Household inventory of HFCSB.
- Specifically: Presence of HFCSB as a categorical variable (≥ 7 vs < 7).
- Define the measurement tool in the methods section. For this example, an audit tool in the home was described. Information on the validity and reliability of the tool should be included or referenced.

Outcome

- Body mass index (BMI)
- Specifically: BMI ≥ 30 compared to the reference BMI category < 30

Study design: Cross-sectional

- The questionnaire on the presence of HFCSB is conducted at the same time as the measurements for the BMI.

Incidence vs prevalence

- Cumulative incidence would be the first measurement of BMI ≥ 30 within a specified time such as across 1 year. The prevalence would be all subjects with a BMI ≥ 30 at one point in time. A cross-sectional study can only evaluate prevalence.

Figure 6. Definitions and study design options of a cross-sectional epidemiologic study: Scenario 1. NOTE: Information from this figure is available online at www.adajournal.org as part of a PowerPoint presentation.

an estimate of the RR in case-control studies where the study design is built on the outcome rather than the exposure and it is a meaningful substitute for a RR. Additional information on the interpretation of binary outcomes from case-control studies has been offered by Kreamer (28) with suggestions on the translation of effect size to clinical practice.

Confidence Intervals

The full description of the potential range for the estimate of the association is conveyed in the confidence interval. The confidence interval provides the information on the results of the statistical test that may either be statistically significant (otherwise known as not due to chance) or not statistically significant (due to chance). In other words, the data do not allow us to rule out the hypothesis that there is no true relationship between the exposure and the outcome. This interval represents the range of values that likely contain the true RR or OR value in the population as a whole and therefore should always be included when reporting a RR or OR. According to classical statistical theory the width of the interval is established by the α level, which is the probability of a type I error. A type I error occurs when the true value of the RR or OR falls outside of the estimated confidence interval. By convention the α level for many two-sided tests is .05 and the confidence interval is set at $(1 - \alpha)$ or 95%. The second characteristic of a confidence interval for an OR or RR is the orientation of this range relative to

| | | | | |
|--------------------------------------------------------------------------------------------------------|--------------------|---------------------------------------------|-----------|------|
| A | | Exposure | | |
| | | Yes | No | |
| Outcome | | | | |
| Yes | a | b | | |
| No | c | d | | |
| <hr/> | | | | |
| B | | Exposure | | |
| | | Categories of HFCSB in the Household | | |
| Model A: Dichotomous Categories | | | | |
| Outcome | | | | |
| Body mass index category | ≥ 7 items | < 7 items | | |
| ≥ 30 | 124 ^(a) | 79 ^(b) | | |
| < 30 | 160 ^(c) | 237 ^(d) | | |
| $OR = \frac{a \cdot d}{b \cdot c} = \frac{124 \cdot 237}{160 \cdot 79} = \frac{29,388}{12,640} = 2.33$ | | | | |
| C | | Category of HFCSB in the Household | | |
| | | Model B: Four Categories | | |
| Outcome | | | | |
| Body mass index category | > 12 items | 7-12 items | 1-6 items | None |
| ≥ 30 | 74 | 50 | 31 | 48 |
| < 30 | 60 | 100 | 120 | 117 |
| <hr/> | | | | |
| OR by category | OR | 95% Confidence interval | | |
| > 12 items | 3.01 | 1.86, 4.85 | | |
| 7-12 items | 1.22 | 0.76, 1.97 | | |
| 1-6 items | 0.63 | 0.38, 1.06 | | |
| None | 1.00 | — | | |

Figure 7. Conceptual and demonstration models of a crude odds ratio (OR) from a cross-sectional study (from Scenario 1: *An investigator wishes to examine the association between the presence of a high-fructose corn syrup beverage (HFCSB) in a household and the prevalence of obesity.*) Note: Hypothetical data for illustration purposes. Panel A: Conceptual model of a crude OR. Panel B: Calculations of a crude OR from Scenario 1. The association between the presence of ≥ 7 or more beverages with HFCSB in the home compared to < 7 on the prevalence of obesity. Panel C: Comparison of an exposure in two categories vs four categories. NOTE: Information from this figure is available online at www.adajournal.org as part of a PowerPoint presentation.

unity or one. If the confidence interval includes one then the conclusion would be that the investigator cannot rule out the possibility that the relationship between the ex-

| |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>Format for presentation x.xx (95% confidence interval [CI] x.xx-x.xx)</p> <p>Examples: Scenario 1 for an odds ratio (OR): OR 2.33 (95% CI 1.64-3.29) Scenario 2 for an OR: OR 0.48 (95% CI 0.32-0.72) Scenario 3 for a relative risk (RR): RR 1.22 (95% CI 1.10-1.36)</p> <p>Description</p> <ul style="list-style-type: none"> ● State the direction of the association (see Figure 4). ● Name the outcome. ● Name the exposure category level. ● Name the reference category. ● Provide the relative risk. The convention is to use two decimal places unless the precision is low in which case use one. ● Provide the 95% CI. ● If the analysis was adjusted for confounding factors include in the statement a notation to that effect. <p>Statistical significance</p> <ul style="list-style-type: none"> ● If a confidence interval does not include one, the association is unlikely to be due to chance, which is otherwise known as statistically significant. |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Figure 8. Template for presenting data gleaned from an epidemiologic study.

posure and outcome estimated in the study is due to chance and thus the estimated relationship would be considered not statistically significant (Figure 5). For other types of statistical tests the relevant comparison would be whether the confidence interval includes zero. The last characteristic is the width of the range which is an indicator of precision or the likelihood of a type II error. There is more confidence that the point estimate is true when there are more subjects in an analysis. If a RR point estimate is 1.4 and the 95% confidence interval is 1.38 to 1.41, this very narrow range suggests a great deal of precision in placing the point estimate at 1.4. But if the confidence interval is 0.36 to 12.81 that point estimate of 1.4 looks quite imprecise and there is little assurance that the true association can be estimated at 1.4. A small sample size will often reduce the confidence and widen the interval. As the width of the confidence interval increases, the lack of precision will affect the ability to reject the null hypothesis because the range is more likely to include one. For information on alternate methods to the classical statistical approach, including a Bayesian framework for interval estimation and interpretation, see Iverson (29).

Sample Applications in Nutrition Research

Following are three scenarios to model public health, clinical, and wellness hypotheses that may support further illustration of epidemiologic methods. (Note: all scenarios are developed with fictitious values for illustration purposes only.)

Cross-sectional Study: Scenario One. *An investigator wishes to examine the association between the presence of a high-fructose corn syrup beverage in a household and the prevalence of obesity.*

| |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>Hypothetical Scenario 2: <i>The association between the intake of fruit and vegetables and the incidence of hypertension.</i></p> <p>Exposure</p> <ul style="list-style-type: none"> ● Fruit and vegetable intake ● The reported intake of servings of fruit and vegetables as ≥ 5 servings/d compared to < 5 servings/d. ● Describe the dietary method used to estimate fruit and vegetable intake, including the tool's validity and reliability. Describe how the summary variable for servings of fruit and vegetables was categorized. <p>Outcome</p> <ul style="list-style-type: none"> ● First incident of hypertension ● Specific levels of diastolic and systolic blood pressure should be defined. ● Methods to determine this outcome may be a direct measurement, self-report, or by the use of blood pressure medication. ● First incidence refers to the first time diagnosis of hypertension over a defined period of time for eligibility into the study. An example would be the incidence of hypertension diagnosed within the past 6 mo. <p>Study design: Case-control</p> <ul style="list-style-type: none"> ● Outcome is hypertension: Identify subjects with a new diagnosis of hypertension within the past 6 months and a control group without hypertension. Retrospectively obtain an estimate of fruit and vegetable intake at a time period in the past. ● Food frequency questionnaires may be posed to ask about previous time periods such as 1 year before enrollment or at a select age. <p>Incidence vs prevalence</p> <ul style="list-style-type: none"> ● Incidence would be the first diagnosis of hypertension. Prevalence would be all subjects with hypertension. This scenario has specified a hypothesis related to incidence cases of hypertension. |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Figure 9. Definitions and study design options of a case-control epidemiologic study: Scenario 2. NOTE: Information from this figure is available online at www.adajournal.org as part of a PowerPoint presentation.

An example of a cross-sectional study addressing this hypothesis is provided in Figure 6. Hypothetical data examine 600 households where an audit was conducted for the presence of high-fructose corn syrup beverage in the home and reference subject's height and weight were measured. The generic two-by-two table for comparison of an exposure and an outcome is presented in Figure 7, panel A. An analysis of the hypothetical data is presented in Figure 7, panel B. An additional analysis illustrates the information that is obtained by examining the exposure in four levels compared to two levels (dichotomous) (Figure 7, panel C). As the results indicate, an association was demonstrated between the highest category and the reference group. However, if the analysis was limited to only a dichotomous variable, the shape of the association would not be visible at the lower two levels of intake.

The crude OR generated from a statistical program using a cross-tabular analysis by the Mantel-Haenszel method would yield an OR of 2.33 (95% confidence interval 1.64 to 3.29) for the hypothetical data. For this crude analysis without adjustment for confounding factors this

Table. Calculation of a crude odds ratio from a case-control study (from Scenario 2: *The association between the intake of fruit and vegetables and the incidence of hypertension*)^a.

| Outcome | Exposure | |
|--------------|------------------------------------------|-----------------|
| | Categories of Fruit and Vegetable Intake | |
| Hypertension | ≥5 servings/day | <5 servings/day |
| Yes | 41 (a) | 199 (b) |
| No | 108 (c) | 252 (d) |

$$OR = \frac{a \cdot d}{b \cdot c} = \frac{41 \cdot 252}{199 \cdot 108} = \frac{29,388}{12,640} = 0.48$$

^aHypothetical data for illustration purposes.
NOTE: Information from this table is available online at www.adajournal.org as part of a PowerPoint presentation.

Exposure

- BMI. Body weight and height obtained from the medical record.
- BMI initially recorded as a continuous variable and later categorized. The example provided examines BMI as a dichotomous variable <30 compared to ≥30.

• Outcome (effect, endpoint, dependent variable)

- A first diagnosis of type 2 diabetes.
- Specifically: Type 2 diagnosis within 10 years – yes or no.

• The outcome of disease needs a diagnosis date for the survival analysis. The method for determining diagnosis should be described.

Study design: Prospective cohort

- Exposure is BMI. Categorized as normal weight or overweight compared to obese and followed for 10 years for the incidence of type 2 diabetes.

Incidence vs prevalence

- Incidence would be a first diagnosis of type 2 diabetes over 10 years, whereas a prevalence study would examine all subjects with type 2 diabetes at one point in time regardless of the date of the diagnosis.

Figure 10. Definitions and epidemiologic study design options applied to Scenario 3: *The association between a body mass index (BMI) of ≥30 compared to the reference BMI of <30 and the incidence of type 2 diabetes in subjects followed for 10 years.* NOTE: Information from this figure is available online at www.adajournal.org as part of a PowerPoint presentation.

estimated association is the same as that generated by the hand calculation of the OR shown in Figure 7, panel B. The summary statement of the results of this analysis would then be “There was a positive association between the presence of ≥7 high-fructose corn syrup beverage items in the home compared to the reference category of <7 on the prevalence of obesity, OR 1.90 (95% confidence interval 1.34 to 2.69).” A template for the presentation of results can be found in Figure 8.

Case-Control Study: Scenario 2. An investigator wishes to examine the association between the intake of fruit and vegetables and the incidence of hypertension.

A

| Exposure | Outcome | | |
|----------|---------|----|-------|
| | Yes | No | |
| Yes | a | b | a + b |
| No | c | d | c + d |

Relative risk = Incidence in the exposed (I_E) compared to the incidence in the non-exposed ($I_{\bar{E}}$): $I_E/I_{\bar{E}} = [a/(a+b)]/[c/(c+d)]$

B

| Exposure | Outcome – type 2 diabetes | | |
|-----------------|---------------------------|-----|-----|
| | Yes | No | |
| Body mass index | | | |
| ≥30 | 365 | 67 | 432 |
| <30 | 116 | 52 | 168 |
| | 481 | 119 | |

Relative risk: $I_E/I_{\bar{E}} = [a/(a+b)]/[c/(c+d)]$
Relative risk: $= [365/432]/[116/168] = 0.84/0.69$
Relative risk: $= 1.22$

Figure 11. Conceptual and demonstration models of a crude relative risk from the analysis of a prospective cohort study (from Scenario 3: *The association between a BMI of ≥ 30 compared to the reference BMI of <30 and the incidence of type 2 diabetes in subjects followed for 10 years.* Note: Hypothetical data for illustration purposes. Panel A: Conceptual model for relative risk. Panel B: Demonstration of the model for a relative risk (Scenario 3). NOTE: Information from this figure is available online at www.adajournal.org as part of a PowerPoint presentation.

The second example (Figure 9) uses the research question of scenario two to demonstrate a case-control design analyzed with an OR. Eligibility for the study is a first diagnosis or otherwise known as incident hypertension within the past 6 months compared to a control group with no current or previous diagnosis of hypertension. The results generated from a statistical program using a cross-tabular analysis would yield an OR of 0.48 (95% confidence interval 0.32 to 0.72), which again would be the same as the manual calculation because there were no confounding factors included in the analysis (see the Table). Following the template for the summary statement the results would be presented as, “There was a negative association between hypertension and the intake of fruits and vegetables at ≥5 servings per day compared to the reference category of <5 servings per day, OR 0.48 (95% confidence interval 0.32-0.72).” This can also worded as a protective effect of the exposure (fruits and vegetable consumption) on the outcome (hypertension).

Cohort Study: Scenario 3. An investigator wishes to examine the association between obesity and the incidence of type 2 diabetes in subjects followed for 10 years.

Scenario three is a prospective cohort study of the as-

sociation between obesity at baseline and the incidence of type 2 diabetes in subjects followed for 10 years comparing BMI ≥ 30 to the reference category of BMI < 30 (Figure 10). A hypothetical database has been created with 600 subjects. Variables for the calculation of the RR include: the exposure (BMI in two categories < 30 and ≥ 30), and the outcome—the incidence of type 2 diabetes (yes/no). There was a positive association between a BMI ≥ 30 compared to the reference group BMI < 30 and the incidence of type 2 diabetes, RR 1.22 (95% confidence interval 1.10-1.36) (Figure 11).

CONCLUSIONS

This monograph has briefly presented essential principles, typical scenarios, hypothetical results, and areas for additional training in the use of epidemiologic techniques common to nutrition research. Appreciating the vitality of a well-defined hypothesis, selecting the optimal study design, appropriately gathering and analyzing the outcomes of interest, taking into account confounding variables, and communicating the oral and written findings of these scientific efforts is paramount to advancing the field and practice of nutrition. It is important to highlight that a thorough understanding of these principles before conducting rigorous nutrition-related research is fundamental. Readers of this monograph are encouraged to build relationships with a multidisciplinary team, including investigators in other areas of nutrition research, biostatisticians, and epidemiologists to strengthen skills in this area and contribute to the publication of quality nutrition research.

STATEMENT OF POTENTIAL CONFLICT OF INTEREST:
No potential conflict of interest was reported by the authors.

References

1. Bushey C, Harris J, Bruemmer B, Archer S, Van Horn L. Publishing nutrition research: A review of study design, statistical analyses, and other key elements of manuscript preparation, Part 1. *J Am Diet Assoc.* 2006;106:89-96.
2. Bushey C, Harris J, Bruemmer B, Archer S. Publishing nutrition research: A review of sampling, sample size, statistical analysis, and other key elements of manuscript preparation, Part 2. *J Am Diet Assoc.* 2008;108:679-688.
3. Harris J, Boushey C, Bruemmer B, Archer S. Publishing nutrition research: A review of nonparametric methods, Part 3. *J Am Diet Assoc.* 2008;108:1488-1496.
4. Harris JE, Gleason PM, Sheean PM, Boushey C, Beto JA, Bruemmer B. An introduction to qualitative research for food and nutrition professionals. *J Am Diet Assoc.* 2009;109:80-90.
5. Gordis L. *Epidemiology*, 3rd ed. Philadelphia, PA: WB Saunders; 2000.
6. Thomas DC. *Statistical Methods in Genetic Epidemiology*. Oxford, UK: Oxford University Press; 2004.
7. Berkman LF, Kawachi I. *Social Epidemiology*. Oxford, UK: Oxford University Press; 2000.
8. Gregg MB. *Field Epidemiology*. 2nd ed. Oxford, UK: Oxford University Press; 2002.
9. Willett W. *Nutritional Epidemiology*. 2nd ed. Oxford, UK: Oxford University Press; 1998.
10. Hill AB. The environment and disease: Association or causation? *Proc R Soc Med.* 1965;58:295-300.
11. Rothman KJ. *Modern Epidemiology*. Boston, MA: Little, Brown and Company; 1986.
12. Schlesselman JJ. *Case-Control Studies: Design, Conduct, Analysis*. New York, NY: Oxford University Press; 1982.
13. Breslow NE, Day NE. *Statistical Methods in Cancer Research. Volume 1—The Analysis of Case-Control Studies*. Lyon, France: IARC; 1980. IARC Scientific Publication No. 32.
14. Kristal AR, Peters U, Potter JD. Is it time to abandon the food frequency questionnaire? *Cancer Epidemiol Biomarkers Prev.* 2005; 14:2826-2828.
15. Schatzkin A, Kipnis V, Carroll RJ, Midthune D, Subar AF, Bingham S, Schoeller DA, Troiano RP, Freedman LS. A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: Results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. *Int J Epidemiol.* 2003; 32:1054-1062.
16. Schatzkin A, Kipnis V. Could exposure assessment problems give us wrong answers to nutrition and cancer questions? *J Natl Cancer Inst.* 2004;96:1564-1565.
17. Dawber TR, Meadors GF, Moore FE Jr. Epidemiological approaches to heart disease: The Framingham Study. *Am J Public Health.* 1951; 41:279-286.
18. Stampfer MJ, Willett WC, Colditz GA, Speizer FE, Hennekens CH. A prospective study of past use of oral contraceptive agents and risk of cardiovascular diseases. *N Engl J Med.* 1988;319:1313-1317.
19. Harrell, FE. *Regression modeling strategies*. New York, NY: Springer, 2001.
20. Body mass index for adults. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/nccdphp/dnpa/bmi/adult_BMI/about_adult_BMI.htm. Accessed March 9, 2009.
21. Koepsell T, Weiss N. *Epidemiologic Methods; Studying the Occurrence of Disease*. Oxford, UK: Oxford University Press; 2006.
22. Hennekens CH, Buring JE. *Epidemiology in Medicine*. Philadelphia, PA: Lippincott Williams & Wilkins; 1987.
23. Breslow NE, Day NE. *Statistical Methods in Cancer Research, Volume II: The Design and Analysis of Cohort Studies*. WHO International Agency for Cancer Research. Publication No. 82. Lyon, France; 1987.
24. Agresti A. *Categorical Data Analysis*. 2nd ed. Hoboken, NJ: Wiley & Sons; 2002.
25. Kraemer HC, Blasey CM. Centering in regression analyses: A strategy to prevent errors in statistical inference. *Int J Methods Psychiatr Res.* 2004;13:141-151.
26. Meinert CL. *Clinical Trials: Design, Conduct and Analysis*. New York, NY: Oxford University Press; 1986.
27. Bland JM, Altman DG. Survival probabilities (the Kaplan–Meier method). *BMJ.* 1998;317:1572.
28. Kraemer HC. Correlation coefficients in medical research: From product moment correlation to odds ratio. *Stat Methods Med Res.* 2006; 15:525-545.
29. Iverson GR. *Bayesian Statistical Inference*. Newbury Park, CA: Sage Publishers; 1984.
30. Kelsey JL, Thompson WD, Evans AS. *Methods in Observational Epidemiology. Monographs in Epidemiology and Biostatistics. Vol 10*. New York, NY: Oxford University Press; 1986.
31. Kleinbaum DG, Kupper LL, Morgenstern H. *Epidemiologic Research: Principles and Quantitative Methods*. Belmont, CA: Lifetime Learning Publications; 1982.