



## SUMMARY

Understanding the strengths and weaknesses of different types of nutrition research studies can help put the latest research findings about diet and disease into proper perspective. Contradictory stories in the media, which generally arise from single studies, contribute to consumers' confusion about what to eat for good health. Failure to appreciate the evolving nature of nutrition research and that some types of research studies carry more weight than do others can contribute to this confusion.

## NUTRITION RESEARCH: WHAT CAN STUDIES TELL US?

There are basically two types of nutrition research studies: observational and experimental. Each approach makes a contribution to elucidating etiologic factors in disease. Observational studies measure dietary intake and disease in groups (e.g., ecologic studies) or individuals (e.g., cross-sectional, case-control, and cohort studies). Among observational studies, cohort studies provide the strongest evidence of a link between diet and disease. In this type of study, dietary information is obtained from individuals in a large population which is followed over time to observe the development of the disease of interest. Cohort studies indicate whether individuals with differing dietary intakes vary in their rates of a specific disease.

Observational epidemiologic investigations are useful for generating hypotheses or critical questions. However, for several reasons, they cannot establish a causal relationship. Reasons include the presence of confounding factors, biases, and the difficulty in accurately quantitating dietary intakes of individuals consuming

self-selected diets. Subjects' inability to accurately recall what they eat, or to estimate portion sizes, as well as variations in food composition and incomplete nutrient databases, can lead to errors in accurately determining nutrient intake in observational studies.

Experimental studies include randomized controlled trials (RCTs) and basic research experiments (e.g., *in vitro* and laboratory animal studies). Basic research experiments can help to identify diet-disease relationships and potential biologic mechanisms. However, *in vitro* and experimental animal findings cannot be readily extrapolated to humans.

The "gold standard" for establishing a cause and effect relationship between diet and disease is well designed RCTs in humans. In this type of study, the investigator has direct control over the conditions of the study such as the difference in level of nutrient intake. RCTs provide strong evidence that increasing intake of dairy foods reduces the risk for osteoporosis and hypertension.

A meta-analysis is a quantitative technique of pooling the results of previously completed studies, preferably those examining the same question and using similar methods (e.g., RCTs) to arrive at a conclusion. The strength of conclusions from meta-analyses varies according to such factors as the quality of studies included.

Rarely do single studies provide evidence of a causal relationship between diet and disease. Although some studies (e.g., RCTs) provide more conclusive evidence than do others (e.g., observational studies), a causal relationship becomes more probable when findings from multiple types of studies are consistent. D

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## INTRODUCTION

The public is increasingly interested in the relationship between diet and health (1,2). This interest is driven in part by media reports of new diet-health findings from single studies. However, findings from single studies rarely provide the final answer and can lead to contradictory advice. This situation contributes to Americans' confusion about how food choices influence their health (1-3).

Part of the public's confusion about diet and health may be explained by their unfamiliarity with and unrealistic expectations regarding the nature of nutrition research. The public may fail to appreciate that findings from a single study are rarely definitive; that the scientific process is not always linear or predictable; that findings from some types of research studies carry more weight than do others; and that biases, confounding factors, and measurement errors in dietary intake can influence study outcomes and their interpretation (4,5,6a). For example, findings from observational epidemiological studies suggest a simple correlation and can generate hypotheses, but they cannot establish or disprove a causal relationship (7-11). In contrast, well-designed, randomized, controlled clinical trials are considered to be the "gold standard" in terms of providing strong evidence for a cause and effect relationship between diet and health (4,7,10,11).

The study of diet-disease relationships is challenging given the complex and changing nature of the diet, including limitations in methods for evaluating dietary intake, and the multifactorial etiology of chronic diseases such as osteoporosis, hypertension, heart disease, and cancer (2,7,8). In addition to the dietary component being studied, other dietary factors as well as a number of other lifestyle and genetic variables influence chronic disease risk (2,7,11). Further, many chronic diseases have a long latent period during which changes in many factors, including diet, may occur. Because of these complexities, evidence for causality is most compelling when the totality of evidence or data from all sources — observational epidemiological studies

and experimental investigations (e.g., randomized controlled trials, human feeding or metabolic, and laboratory animal studies) — is consistent and the association is biologically plausible (6a).

This *Digest* reviews various types of nutrition research studies, both observational epidemiology and experimental or controlled investigations and what the findings from these studies can and cannot tell us. Also, methodological considerations such as those related to dietary assessment are discussed.

## TYPES OF NUTRITION RESEARCH STUDIES

### **Observational Epidemiological Studies.**

Epidemiologic research seeks to expose potential associations between diseases and diet, lifestyle factors, or other variables within populations. Most epidemiological research is observational (2,4). Observational studies obtain data measured in groups (e.g., ecologic studies) or individuals (e.g., cross-sectional, case-control, and cohort studies) (6a,7,8,11). The following types of observational studies are used to determine potential associations between diet and disease.

■ *Ecologic (Correlation) Studies.* In ecologic studies, disease morbidity or mortality rates in populations, often in different countries, are compared with specific dietary intakes based on food disappearance data (2,7,8). A major strength of ecologic studies is the typically large contrasts in dietary intake and disease outcome which helps to show a link between nutritional exposure and risk of disease. However, problems of correlation studies include the potential for confounding factors and the crude assessment of dietary intake by food disappearance data. Confounding factors such as other dietary components, lifestyle factors, or ethnicity can make it impossible to distinguish between a response to treatment and some other factor. Because food disappearance data do not correct for losses (e.g., in transport, storage, preparation, or spoilage), intake tends to be overestimated. Ecologic studies are useful in generating hypotheses, but they are unable to provide any conclusions regarding diet-disease relationships (2,7,8).

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*Understanding the strengths and weaknesses of different types of nutrition research studies can help health practitioners put contradictory diet-disease findings into perspective, alleviate the public's concerns, and provide appropriate advice about what to eat for good health.*

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■ *Cross-sectional Epidemiological Observations (or prevalence studies).* In cross-sectional studies, nutritional exposure and disease status (or a biologic measurement of disease) are measured in individuals in a defined population at one point in time (2,4,6a,7,8). Cross-sectional studies have found that individuals with low bone mineral density (12) or high blood pressure (13) are those with low calcium intakes. This type of observational study can examine a large sample size and is useful for estimating links between key risk factors and disease among individuals. However, an important weakness of cross-sectional studies is that it is unknown whether diet may have been altered in response to diagnosis or initial symptoms of disease (8). Also, limited variation in usual dietary intake among individuals in a particular population, large within-person variation in dietary intake, and inaccuracies in dietary survey methods make it difficult to establish correlations between diet and disease. Cross-sectional studies, like ecologic studies, are a relatively weak method for assessing diet-disease associations (2).

■ *Case-Control Epidemiological Observations.* In this type of observational study, a group of individuals with a particular disease (cases) and a group free of the disease of interest (controls) are examined for differences in dietary (or other lifestyle) factors (3,7,8). For example, case-control studies are designed to answer such questions as, "Do persons with osteoporosis (case subjects) consume diets that differ from those consumed by individuals without this disease (control subjects)?" Case-control studies are relatively inexpensive, efficient, yield results fairly quickly, and require fewer individuals than do cohort investigations (2,7,8). However, similar to cross-sectional studies, a major weakness is the possibility that case subjects, because of their disease, will recall their dietary intakes differently than do control subjects (i.e., recall bias). In addition, choosing appropriate controls is always biased unless they are a random sample of the population (11).

■ *Cohort Studies (or long-term follow-up).* Among observational study designs, cohort studies provide the most reliable information (2). Cohort studies obtain dietary (or other) information on individuals in a large population (cohort) and follow the

population to observe who does and does not develop the disease of interest (2,3,6a,7,8). This type of study can help demonstrate whether individuals with differing dietary intakes vary in a risk factor for or their rates of developing a specific disease. For example, cohort studies can help answer the question, "Do persons with low calcium/dairy food intakes develop osteoporosis more frequently or sooner than those who meet their calcium needs?"

Cohort studies avoid many potential sources of methodological bias associated with case-control studies (6a,7,8). Because dietary intake information is measured prior to the disease onset, the illness cannot influence dietary recall. Cohort studies also allow repeated assessments of diet over time and examination of the effects of diet on more than one disease simultaneously (7). The primary disadvantage of cohort studies is that such studies are a long, expensive, large-scale undertaking. If a disease has a low incidence and a long induction period, a large cohort must be followed over a prolonged period of time (2,3,7,8). As a result, attrition may be a problem which can bias the results (2,11). In many cohort studies (e.g., Framingham Heart Study), dietary information is measured only once at baseline. It cannot be assumed that diet remains unchanged throughout the duration of the study (2). Also, because of the large sample size, less detailed and less accurate dietary information may be obtained than for some case-control studies.

Although associations identified from observational epidemiological investigations are useful for generating hypotheses or critical questions, the researcher cannot draw conclusions regarding causality, no matter how carefully the study was conducted (3,4,6a). Confounding factors and potential biases in observational studies may cause an association between diet and disease to be coincidental (4,6a, 14-16). Confidence that a relationship in observational studies might be causal depends on the consistency of findings in different populations and with different methods and studies, the dose response relationship, biological plausibility, and consistency with corresponding animal studies (10).

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*Observational epidemiological studies generate hypotheses regarding diet and disease, but by themselves are unable to establish a cause and effect relationship.*

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A recent review of observational studies examining calcium, dairy products, and osteoporosis found a significant positive association between calcium intake and bone health in approximately three-quarters of 86 observational trials examined (12). The investigator attributed the failure of the remaining one-quarter of studies to find a positive relationship to the inability to control for potential confounding factors, biases, and methodological weaknesses in accurately estimating calcium intake (12,17). As discussed below, stronger support for calcium's beneficial role in bone health comes from investigator controlled studies (12).

**Experimental Studies.** These studies include randomized controlled clinical trials and basic research experiments (e.g., in vitro and laboratory animal studies) (4,6a,7,10,11).  
 ■ *Randomized Controlled Trials (clinical trials).* Randomized controlled trials (RCTs) in humans are considered to be the "gold standard" for evaluating a dietary hypothesis and providing evidence of causality between diet and disease (2,6a,7,10,11,14). In this type of experimental study, subjects are randomly assigned to either a control or an experimental group. The experimental group then receives a treatment or intervention (e.g., increased calcium intake) and the outcome (e.g., bone mineral density) is compared to that of the control or placebo group (2,4,6a,8). Ideally, RCTs are double-blind, meaning that neither the subject nor the investigator is aware of who is receiving the test substance or the placebo (4). However, blinding is usually difficult to achieve in food-related research. In RCTs, the investigator has direct control over the difference in level of nutrient intake. These studies can also achieve a degree of control of confounding factors that is impossible to achieve with any observational study. However, RCTs are expensive, a long duration may be necessary to achieve results, and compliance may be a problem, particularly in RCTs of diet.

Human feeding trials such as some metabolic studies and multicenter

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feeding investigations use a randomized controlled design to establish causal relationships between diet and disease (2,6a,6b). In well-controlled metabolic studies, subjects are randomly assigned to a control diet or a test diet, or sometimes, to a random sequence of control and test diets (i.e., cross-over design). In cross-over studies, subjects serve as their own controls. Subjects are fed precisely measured diets in which one or more components are varied and the effect on a biological variable or risk factor is measured. Metabolic studies typically are carried out in a research setting. Because adherence is high, these studies can quantify the effects of a few dietary constituents on one or more outcome variables. However, these studies generally include a small number of participants (5 to 25) from a narrowly defined population and are of short duration (6a). If larger sample sizes are needed to achieve adequate statistical power, successive cohorts can be studied using the same protocol. Alternatively, a multi-center randomized feeding design can be followed.

An example of a multi-center feeding study is the DASH (Dietary Approaches to Stop Hypertension) trial (6b,13,18,19). This trial demonstrated that a diet rich in low fat dairy products, fruits and vegetables has an unequivocal beneficial effect on blood pressure, particularly in individuals with established hypertension. DASH used a sufficiently large sample size (459 adults) at four centers to allow adequate representation of different groups such as women, minorities (e.g., African Americans), and individuals with and without hypertension, as well as to detect blood pressure reductions of public health relevance (18,19). Also, because the blood pressure-lowering effect of single nutrients may be too small to detect in small-scale studies, the DASH trial tested the effect of dietary patterns on blood pressure.

Compared to observational studies, investigator-controlled diet studies provide stronger, more consistent evidence of a causal relationship between diet and disease (12). As mentioned above, three-quarters of 86 observational

studies examined found a significant positive association between calcium intake and bone health (12). However, a critical review of 52 investigator controlled calcium intervention studies, including RCTs, by the same researcher revealed that all but two studies showed improved bone health at high calcium/dairy intakes (12). Failure of the two studies to demonstrate a beneficial effect of increased calcium/dairy is explained by the control group's relatively high calcium intake in one study, and the inclusion of early postmenopausal women, whose bone loss is mainly due to estrogen withdrawal and less to nutrition, in the second study (12).

■ **Basic Research Studies.** Basic research studies of the effects of diet on disease include *in vitro* (test tube) and experimental animal investigations (4,5,6a,7,8). These types of studies help to confirm observations and provide insights into biologic mechanisms. Compared to human investigations, basic research studies are less expensive and can be carried out in a relatively short period of time (6a). However, findings from *in vitro* and experimental studies cannot be extrapolated to free-living humans.

**Meta-analysis.** A meta-analysis is a formal statistical technique of systematically combining the results from separate but similar previously completed studies to yield overall conclusions about a hypothesis (4,7,20,21). Unlike narrative scientific reviews of the literature, meta-analyses provide a quantitative synthesis of available data. The technique is best used when examining studies addressing the same question and employing similar methods to measure relevant variables (4). Meta-analysis can be a valuable tool to aggregate relevant findings across studies and help to explain differences among studies. However, the strength of conclusions from meta-analyses can vary according to such factors as the type of studies being analyzed (i.e., observational versus RCTs), criteria for inclusion of studies, and statistical methods used (4,8,20-23).

For example, a meta-analysis of observational studies revealed a very weak association between

increasing dairy food intake and blood pressure (24). However, a subsequent meta-analysis of the same studies demonstrated that the effect was actually 30 times greater than estimated in the first analysis (25). Compared to the original meta-analysis (24), this revised meta-analysis corrected for several methodological errors and used more stringent inclusion criteria and statistical methods than did the original meta-analysis (25). Likewise, the quality of meta-analyses of RCTs of calcium/dairy foods and hypertension varies (26,27). A meta-analysis of RCTs revealed considerable heterogeneity in the blood pressure response to increasing calcium intake (26). However, when the same investigators updated this meta-analysis to include DASH and other clinical trials and when more stringent inclusion criteria and rigorous statistical methods were followed (27), the source of calcium was found to account for much of the heterogeneity in blood pressure (27). The updated meta-analysis demonstrated that the blood pressure lowering effect of dietary calcium (e.g., dairy foods) was more consistent than that of calcium supplements (27).

### **DISCREPANCIES BETWEEN OBSERVATIONAL AND RANDOMIZED CONTROLLED TRIALS: DIETARY ASSESSMENT**

Discrepancies in diet-disease relationships between observational studies and experimental investigations such as RCTs are due in large part to the weaknesses of methods available to assess dietary intake in observational studies (6a). In RCTs, nutrient or food intake can be measured with a relatively high degree of accuracy because intake is controlled and nutrient composition of menus can be verified by chemical analysis, as was done in the DASH trial (6a). In contrast, self-reported intakes by food records, 24-hour dietary recall, and food frequency questionnaires used in observational studies are susceptible to inaccuracies and biases (7,28-33). These include errors attributed to memory gaps, inability to

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*A single study can rarely stand alone as scientific confirmation of a hypothesis. Rather, findings from multiple studies, both observational and experimental, taken together, can significantly contribute to our understanding of diet-disease relationships.*

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judge portion sizes, and over- and under-reporting of food intake.

Although currently no dietary assessment method is free of measurement error (2,28), the methods differ in their strengths and limitations (7,28,33). Methods such as 24-hour dietary recall rely heavily on subjects' memory and their ability to accurately characterize food intake (7,33). Food records (i.e., foods and amounts are recorded at the time they are eaten) do not rely on memory, but require individuals to be highly motivated and literate, which can introduce bias toward including educated persons (15,29). Also, food records may need to be kept for a sufficient length of time to accurately indicate intake (34). A number of factors including day-to-day variability in dietary intake, subjects' compliance, and skills of the interviewer can influence the reliability of methods used to assess dietary intake (28). Also, variations in food composition and incomplete nutrient databases can introduce errors when converting foods into nutrients (28). In addition, the bioavailability of nutrients and the potential for nutrient interactions can make it difficult to establish diet-disease relationships in observational studies (8,17,35,36).

## CONCLUSION

Different types of nutrition research studies vary in their strengths and weaknesses. Rarely can a single study provide evidence of a causal relationship between diet and disease (6a).

A causal relationship between diet and disease becomes more probable when relevant data from several different types of studies are consistent (2,6a,8). Putting research findings into context and taking a moderate approach to communicating new findings can help the public better understand the evolutionary nature of nutrition science and determine whether or not a dietary change is justified. D

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## Coming Next Issue:

# CALCIUM-FORTIFIED FOODS: IS THERE A REASON FOR CONCERN?

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