The Science of Food and Health: Nutritional Epidemiology

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Now you know *what* to eat.
Why ask why?

Know the rationale for eating specific foods and avoiding others, empowering you to...

- Think for yourself
- Make informed choices
- Convince yourself to seek out and stick with the best diet for you
- Develop willingness to try new foods that are good for you
- Resist being swept along with each new fad
- Gain skills to critically evaluate new recommendations
- Reduce enticing foods that are detrimental
- Satisfy curiosity

Learning “Why” is hard work - but worth the effort

Requires understanding new concepts

Requires thinking about evidence

Requires making cost / benefit judgments

Not everyone has the background or interest to do the necessary work

However, you don’t need to master everything

As long as you get the gist, the data presented as we move through foods and nutrients will make more sense

Epidemiology

“The science that studies patterns, causes and effects of health and disease conditions in defined populations“

Etymology: closely related to “epidemic“

- epi (Gr.) “upon, among“
- demos (Gr.) “people, district“
- logos (Gr.) “study, word, discourse“

Nutritional epidemiology

A branch of science that focuses on the relationship of ***diet*** to health and disease

NE is conducted in a variety of settings, including international agencies (WHO), NGOs, national agencies (NIH, CDC, FDA, NSF, USDA), state agencies (DHHS), local health departments, research institutes, schools, non-profit organizations

Commercial interests (food producers, trade associations) also conduct studies, although require more scrutiny to check for potential bias

Outcomes and factors

Outcomes ("effects")

- Longevity
- Disease onset
- Weight change
- Blood pressure change
- Biochemical change (e.g., cholesterol level)

Factors ("causes")

- Controlled conditions
  - Prescribed diet
  - Medication
- Spontaneous
  - Smoking habit
  - Exercise
  - Free-living diet

Level of understanding

Association

- Correlation between factor and outcome found, but cause-and-effect relationship not established

Causal

- Linkage from cause to effect established, but mechanism remains unknown

Mechanistic

- Cause and effect relationship established, and the mechanistic path from cause to effect is identified

Getting to the mechanistic level often requires collaboration between basic and epidemiologic research

Studies

The purpose of studies is to infer causes, draw conclusions, and support policy-making

- Pose question: for example, does increasing dietary fiber reduce heart disease mortality?
- Plan and conduct research
- Analyze data: evidence says "Yes"
- Policies: Promote increased fiber consumption
  - Individual, through education and promotion
  - Societal, through regulation and incentives

Types of studies

Basic research ("bench research")

Randomized controlled trials

Cohort (observational) studies

Basic research

Genomics

- Look for genes that are associated with diseases and susceptibility to exposures

Environmental factors

- Evaluating exposures for possible disease causation and progression by study in laboratory animals

Mechanisms of benefit or harm

- How excessive fructose consumption affects metabolism

Randomized controlled trials

"Gold standard" of research

Subjects allocated to different treatment arms by randomization

Outcomes of treatment arms evaluated by statistical tests

Statistically significant results allow us to infer causality

Drawbacks with respect to nutritional epidemiology

- Long, expensive, intrusive
- Short-term studies inconclusive
- Rarely practical in nutritional research
- False positives and false negatives

Randomized Clinical Trials

Lyon Diet Heart Study (France)

- 300 subjects with existing heart disease (secondary prevention)
- Followup: 5 yrs

PREDIMED (Spain)

- 8000 subjects at risk for heart disease (primary prevention)
- Followup: 5 yrs

Both studied Mediterranean Diet

Each showed significant and meaningful benefit in reducing coronary heart disease compared to control diet

Observational study

The purpose is to relate *outcomes* (mortality rate, disease onset) to *factors*

- Does greater fiber consumption reduce mortality?

Select a sample of individuals with some well defined common attributes (nationality, occupation, age, etc)

Record factors

Determine outcomes

Analyze relationship of outcomes to factors

Observational studies

Record as many *factors* as feasible and likely to affect outcome

- Age, weight, height, blood pressure, educational level, exercise level, blood tests
- These can be taken into account in multifactor analysis

Obtain dietary history

Obtain biochemical markers, where feasible (salt excretion, lipoprotein blood level)

Follow up to determine *outcomes:* death, cause of death, weight change and/or onset of specific diseases

Diet history collection

Precise

- Provide meals from a research kitchen
- Feasible only for short-term, focused uses

Approximate

- Food diary
- 24-hr food recall
- Food frequency questionaire: entered by subject on scan form
- Reasonable agreement among all three
- FFQ feasible in more situations

Food frequency questionnaire

BREADS (include use as toast and sandwiches)	Never or rarely	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	Standard Serving Size	Standard	1/2 or less	1 1/2 or more
White bread, rolls, buns, or French bread	○	○	○	○	○	○	○	○	○	2 slices or 1 bun/roll	○	○	○
Whole grain bread, rolls, buns, or oatmeal bread	○	○	○	○	○	○	○	○	○	2 slices or 1 bun/roll	○	○	○
Corn bread, Johnnycake	○	○	○	○	○	○	○	○	○	2 slices or pieces	○	○	○
Other breads, bagels, biscuits you eat? (*please write them in - use* CAPITAL *letters*):													
1. ____________	○	○	○	○	○	○	○	○	○	2 slices or pieces	○	○	○

Converting FFQ entries to study factors

How you use the FFQ entries depends on the questions posed in the study

A study evaluating at the food level may use food frequencies directly as obtained

A study evaluating at the nutrient level requires breaking each food into its components

- Bread is partitioned into fat (saturated, monounsaturated, polyunsaturated), protein (amino acids), carbohydrate (sugar, starch, fiber), vitamins, minerals, phytochemicals
- Partitioning uses standard reference tables for food and beverage

National Nutrition Database for Standard Reference

USDA Branded Food Products Database
Release v0.0 March, 2018

Full Report (All Nutrients) 45102292, BREAD, MADE WITH WHOLE GRAIN, UPC: 071301047179

Nutrient	Unit	Data points	Std. Error	2.0 SLICES 57g	1 Value Per100 g
Proximates					
Energy	kcal	--	--	150	263
Protein	g	--	--	5.00	8.77
Total lipid (fat)	g	--	--	2.00	3.51
Carbohydrate, by difference	g	--	--	28.00	49.12
Fiber, total dietary	g	--	--	2.0	3.5
Sugars, total	g	--	--	3.00	5.26
Minerals					
Calcium, Ca	mg	--	--	200	351
Iron, Fe	mg	--	--	1.58	2.77
Sodium, Na	mg	--	--	280	491
Vitamins					
Vitamin C, total ascorbic acid	mg	--	--	0.0	0.0
Thiamin	mg	--	--	0.000	0.000
Riboflavin	mg	--	--	0.136	0.239
Niacin	mg	--	--	1.754	3.077
Vitamin A, IU	IU	--	--	0	0
Lipids					
Fatty acids, total saturated	g	--	--	0.000	0.000
Fatty acids, total monounsaturated	g	--	--	0.000	0.000
Fatty acids, total polyunsaturated	g	--	--	0.502	0.880
Fatty acids, total trans	g	--	--	0.000	0.000
Cholesterol	mg	--	--	0	0

Observational study analysis

Evaluate outcomes with respect to the "risk factors" (demographic, personal and diet data) using statistical tests

- Example: Heart attack rate is higher in people eating more processed meat

Statistical significance indicates association between risk factors and outcome

Association does not prove causation

- *Achilles' heel* of observational studies
- Validate by replication in different settings
- Identifying plausible biological mechanisms provides support

Major observational studies

Nurses Health Study (NHS)

- 100K
- 30 yrs

Health Professional Followup Study (HPFS)

- 50K
- 25 yrs

Physicians' Health Study (PHS)

- 50K
- 25 yrs

NIH-AARP Diet and Health Study (AARP)

- 500K
- 10 yrs

European Prospective Investigation into Cancer and Nutrition (EPIC)

- 500K
- 18 yrs

Swedish Mammography Cohort (SMC)

- 61K
- 22 yrs

Adventist Health Study (AHS)

Health Survey for England (HSE)

National Health And Nutrition Examination Study (NHANES)

Statistics

The major tools used in nutritional epidemiology are statistical methods

Human judgment relies on hunches, intuition, and common sense - which are subject to bias

Statistics provides objective methods and tools for planning research studies and and evaluating their results

Planning

- Identify target population; formulate sampling strategy

Descriptive statistics

- Summarizing observed data
- Shape of distribution (e.g., classical “bell-shaped”)
- Central measure (e.g., mean) and dispersion (e.g., standard deviation)

Inferential statistics

- Drawing conclusions about relationship between factors

Sampling

When a population to be studied is large, it's costly to obtain values from every subject

An appropriately-taken sample can be sufficient to answer the study question

A random sample is the most desirable

- Statistical methods strictly applicable

Much effort goes into eliminating bias in the sampling

Necessity to over-sample under-represented subgroups

Descriptive statistics

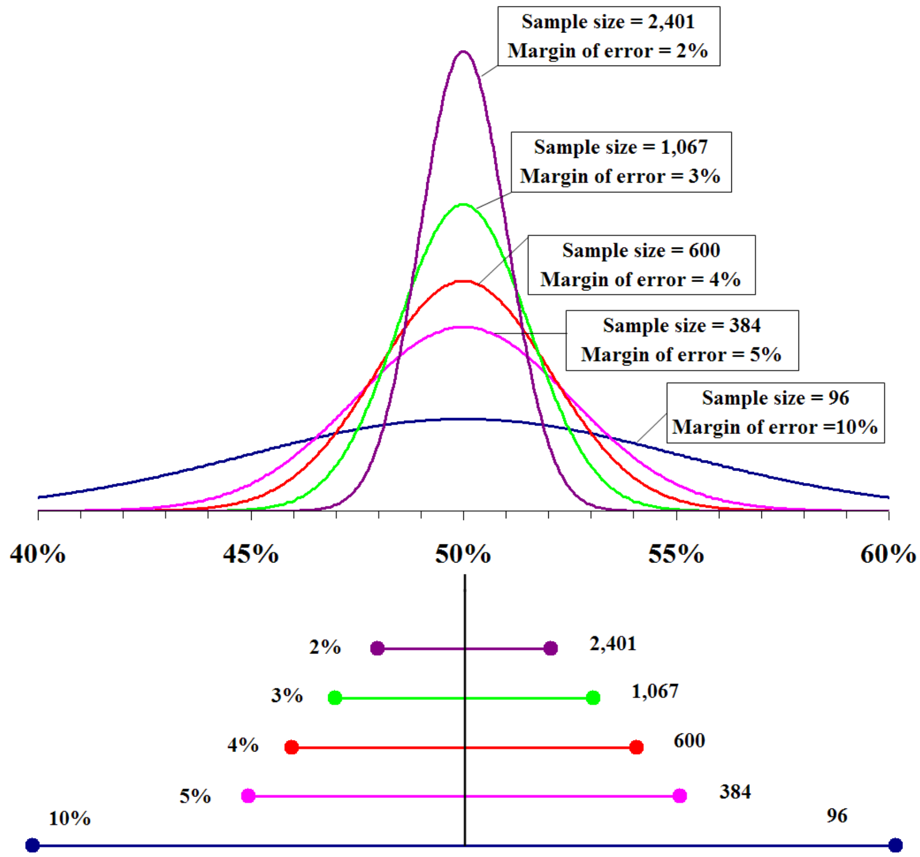
Summarizing observations in structured way

Example: Sodium intake in U.S. population (NHANES)

- Population: 320M; sample: 8K (1/40K)
- Random sample: representative
- Mean sodium intake: 3,480 g/day (±605 SD)
- Quartiles: [2874,3355,3933] - 1st, median, 3rd
- Approximately symmetric about mean

Margin of error

- We seek to find the true value of a parameter in a population
  - How many will vote for a candidate in an upcoming election?
- It's too costly and difficult to poll every member of the population, so we sample
- Margin of error provides a measure of how closely the observed value (e.g., a mean) reflects the true value of the sampled population
- AKA confidence interval, confidence limits
- Quoted at a specified "confidence level"
  - 95% is an oft-used level; it's 100% minus the specified error (5%)
- Margin of error depends on sample size



Drawing Conclusions: Inference

"There's something going on here. We've got to get to the bottom of it" - or maybe not!

Put forward a proposition, collect data to test it, chose a statistical model that applies, and either confirm or reject it

Conclusions can guide future actions and policies

- If increasing amounts of fiber in the diet lowers one's rate of heart attacks, diabetes onset, and death, without offsetting adverse effects, one would give strong consideration to eating as much fiber as practical

Inference making

Similar to trying a crime suspect

Four possible outcomes:

- Defendant guilty, found guilty
- Defendant innocent, found innocent
- Defendant guilty, found innocent (ERROR! Criminal getting off)
- Defendant innocent, found guilty (ERROR! Innocent punished)

Goals:

- Maximize rates of correct conclusion
- Minimize error rates, according to impacts of the different types of error
- Determine magnitude and meaningfulness of effects

Hypothesis testing

Formal name for process of making inferences

State the null hypothesis

- There is no relationship between fiber intake and onset of coronary heart disease

State the alternative hypothesis

- There is a relationship …

Analyze data with appropriate model

Likelihood of observed outcome or more extreme due to chance alone

- Likely: Do not reject the null hypothesis
- Unlikely: Reject the null hypothesis → Accept alternative hypothesis

Hypothesis testing

Determine the probability that the observed result could have happened by chance

- Calculated for the specific data model

If that probability is sufficiently low, declare that the observed result probably did not happen due to chance → "statistically significant"

How low is "sufficiently low"?

- Depends on the consequences of false results
- Conventional level is 1 in 20 (5%)

Known as the "p value"

Example of hypothesis testing

Situation: Coin tossing

Hypothesis: The coin is fair, i.e., equal probability of heads and tails

Alternative hypothesis: The coin is biased, i.e., more likely to land heads than tails, or vice versa

Data: Toss the coin 10 times. It lands 'HHHHHHHHHH'

Model: Tossing a fair coin, the probability of landing all heads (or all tails) is 2 in 2^{10}, or 2 in 1024

Result: Observed value would occur only 0.2% of the time

Conclusion: An event with 0.2% occurrence is a rare event by chance alone. Therefore, we reject the null hypothesis of a fair coin and accept the alternative that the coin is biased.

Discussion: Rejecting the null hypothesis is not declaring certainty; rather it points to fruitful areas for further exploration. Repeating the experiment and obtaining similar results lends more credence. Having a plausible cause → effect mechanism bolsters conclusion (coin is found to have been filed).

Hypothesis testing: Walnut feeding experiment

Sabaté *et al* - NEJM - 1993

18 healthy men fed a cholesterol-lowering diet for 8 weeks in a research kitchen at Loma Linda University; 30% calories from fat

During 4 of the weeks, 20% of the calories came from walnuts, about 3 oz / 2500 Cal

During the other 4 weeks, no walnuts were consumed

Cross-over design, so each man was on both arms of the experiment, with and without walnuts, the order randomly assigned and stratified

Walnut feeding study

Null hypothesis: Eating 3 oz of walnuts a day for 4 weeks has no effect on LDL

Alternative hypothesis: Eating 3 oz of walnuts a day for 4 weeks modifies LDL

Measures: LDL levels after walnut consumption and after control diet

Cholesterol vs. walnut consumption

LDL after control diet: 112±16 (mean±SD)

LDL after walnuts: 94±17

Difference in LDL: -18

$p < 0.001$ (t-test)

"A difference this great would occur by chance 1 in a thousand or less; null hypothesis is rejected"

Conclusion: Eating 3 oz of walnuts a day for 4 weeks lowers LDL cholesterol

Survival analysis

Area of statistical analysis developed for actuaries (life insurance professionals)

- Rate individuals, set premiums
- Base predictions on risk factors (BP, smoking)

Duration of time until an event happens

- Death
- Onset of disease
- Relapse from remission

Wide applicability

- Health - longevity, disease onset, remission duration
- Industrial - Failure of component

Survival analysis - example

Mortality in British physicians re: smoking

Conducted by UK epidemiologist Richard Doll

Study included 35,000 male doctors

Study began 1948, last follow-up 2000

25,000 died, 5,000 remained alive, 4,000 withdrew

Survival analysis - example

What question are we trying to answer?

- What is the universal relationship between smoking and mortality?

What answer are we going to get?

- What is the relationship between smoking and mortality in British male physicians?

Why British male physicians?

- Available, committed

How generalizable are the results?

- This is a troublesome aspect of such studies

Cohort: Doctors aged 30-39

Doctors born 1921-1930: 7,385

1,713 never smoked up to age at entry

- 51 had died by age 50 (3.0%)
- Average annual mortality = 3.0%/15 yr = 0.2%/yr (2.0/1000/yr)

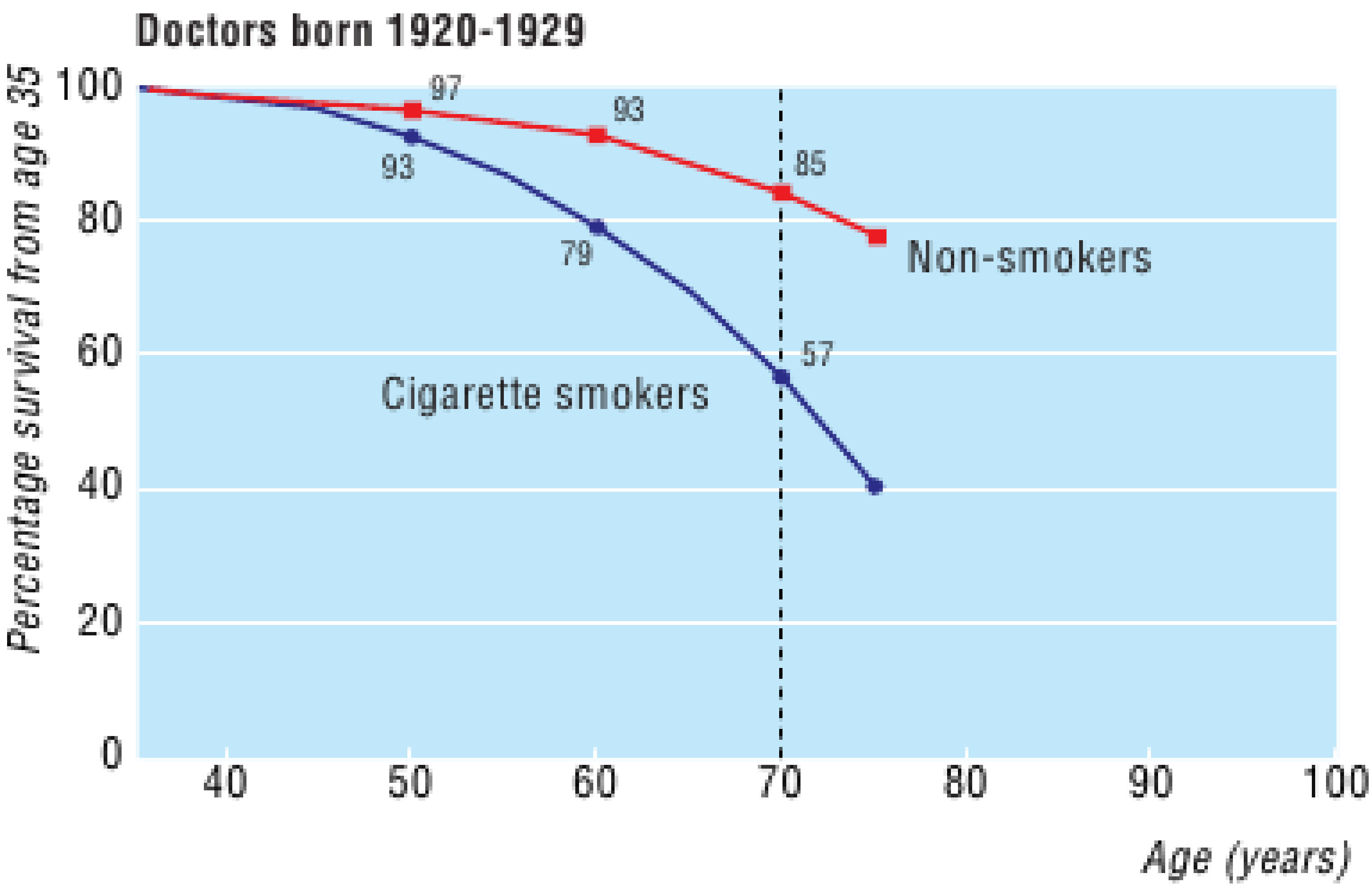
2,252 currently smoked at entry

- 158 had died by age 50 (7.0%)
- Average annual mortality = 7.0%/15 yr = 0.48%/yr (4.8/1000/yr)

3,420 former smokers

- Excluded from analysis

Longevity UK Doctors at Age 35



Mortality rates and ratios

Mortality rates

- Current smokers: 4.8/1000/yr
- Never smokers: 2.0/1000/yr

Mortality ratio (AKA relative risk, risk ratio, hazard ratio)

- MR = RR = HR = 4.8/2.0 = 2.4
- RR > 1: factor has adverse effect on mortality
- RR < 1: factor has beneficial effect on mortality
- RR = 1: factor has no effect on mortality

Interpretation

- "Current smokers at age 35 die 2.4 times as fast in the next 15 years compared to never-smokers"
- "Current smokers have 140% higher mortality rate than never-smokers"

Mortality comparisons: Statistical methods

Standard methods exist to compare mortality of group A (e.g., smokers) to group B (e.g., never smokers)

Strictly applicable only when subjects assigned by randomization

- Not the case in observational studies

Widely used in observational studies with assumption that exposure being studied (e.g., smoking) was not related to another exposure that could have affected outcome (e.g., physical activity)

Great care needed to validate that assumption

Methods exist to separate effects of multiple factors simultaneously (proportional hazards multiple regression)

How meaningful are mortality ratios?

Pretend you are the Surgeon General

- You have to make decisions on policy and advise the public

Smoking increases mortality by 140%

Is this a real difference, or could it have occurred by chance?

Assuming this is a representative sample from a larger population, what generalization can we make?

- What is the effect of smoking in all British males? in all males globally? in men and women?

Relevance of study results

Statistical aspect

- Statistical theory provides us guidance on the reliability of the results we have observed – often the easiest aspect to deal with

Sampling aspect

- How representative is the sample we observed of the underlying population to which we would like to apply our results?

Effect size

- Is the result large enough to be of practical importance, or is it statistically significant but biologically trivial?

Measurement errors

- Dietary history methods are subject to error
- Error can be minimized by various means: biomarkers, different methods of collecting diet history
- Errors tend to reduce or obscure effects, not exaggerate or suggest false effects

Statistical aspect of reliability

Statistical theory provides reliability guidelines

Margin of error, or "95% confidence interval"

- Smoking: 1.73 to 3.21 (+73% to +221%)
- With 95% confidence, the "true value" of the mortality ratio lies within that interval

A mortality ratio of 1.0 is the ratio of "no effect"

- Exposed and unexposed subjects have the same mortality rate
- If 1.0 falls within the 95% confidence interval, we are unable to declare a significant difference between the exposed and unexposed subjects in the outcome

A mortality ratio whose 95% confidence interval does not include 1.0 is significant

- Mortality is significantly related to smoking

Mortality ratio with confidence interval

Confidence interval depends on number of subjects as well as the effect size

- The larger the number of subjects, the smaller the confidence interval, and the more precise the estimate of the true effect of the exposure

More subjects are required when we are trying to detect small effects

Extremely large samples may find statistically significant results that are not practically meaningful

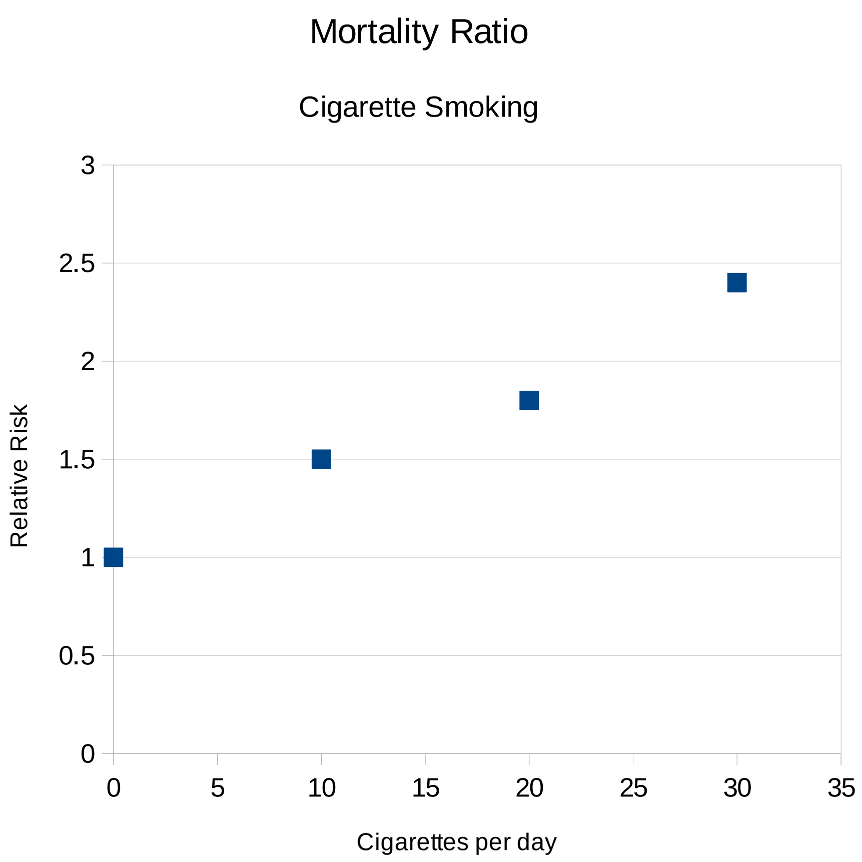
Dose-response: Smoking

Addressing the question: "Is smoking all-or-none, or does the harm increase with dose (number of cigarettes smoked per day)?"

Reference (comparison) is non-smokers

Cigarettes/day	0	1-14	15-24	>24
Mortality rate	19	29	35	45
Mortality ratio	1.0	1.5	1.8	2.4

Dose-response analysis: graphical



How does outcome (mortality, disease incidence) relate to level of exposure to factor?

Smoking has a direct (adverse) effect on mortality

Quantiles

Grouping subjects into equal-sized groups

- Halves – 2 groups
- Tertiles – 3 groups
- Quartiles – 4 groups
- Quintiles – 5 groups
- Deciles – 10 groups

Comparisons are made between each quantile and the reference group

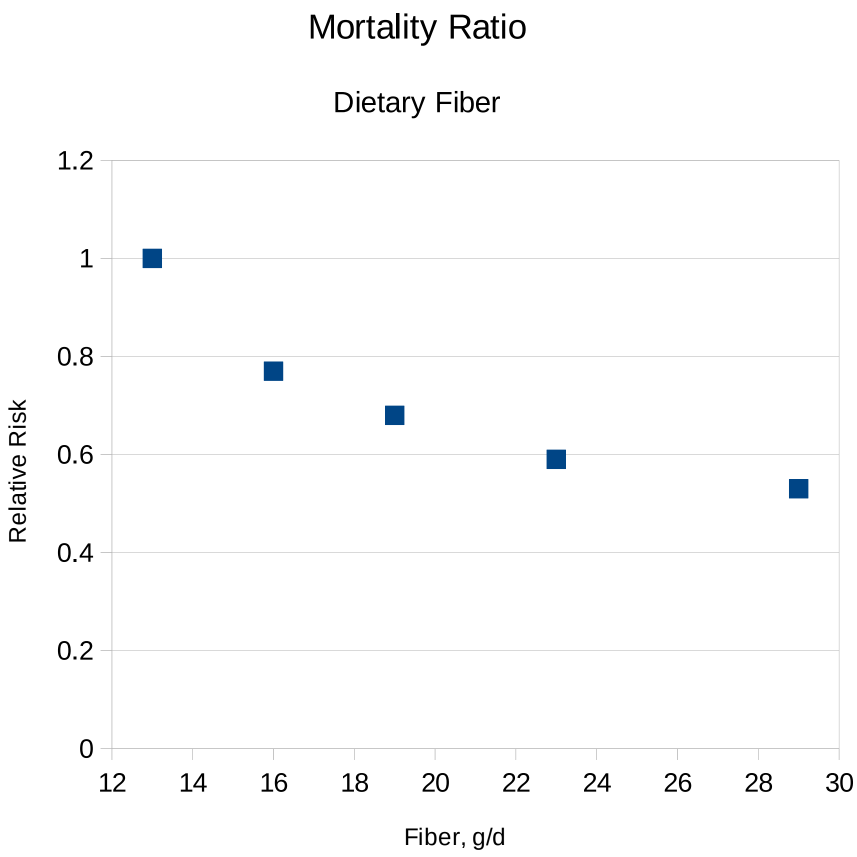
- In this example, subjects are groups in quintiles
- Reference group is quintile 1, the lowest fiber intake
- RR for each other group is its comparison to the first quintile

Dietary fiber and mortality					
Quintile	1	2	3	4	5
Fiber, g/d	13	16	19	23	29
RR	1.00	0.77	0.68	0.59	0.53

Dose-response analysis

How does outcome (mortality, disease incidence) relate to level of exposure to factor?

Dietary fiber has a inverse (beneficial) effect on mortality



Mortality and dietary fiber

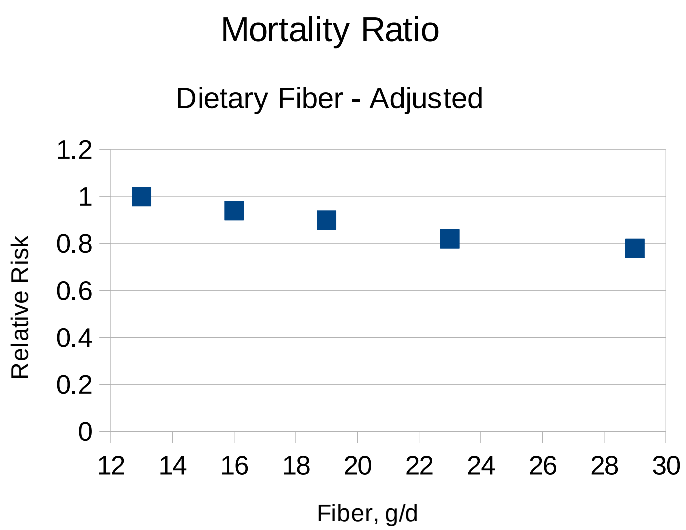
You can cut your mortality rate in half just by doubling your fiber intake?!?

But wait! Those eating less fiber are more likely to smoke cigarettes, are less likely to exercise, have higher body mass index, i.e., have additional risk factors

How do you take the effects of these co-factors into account?

- Multivariable statistical methods
- Proportional hazards multiple regression

Dose-response, adjusted for risk factors



Dietary fiber and mortality					
Quintile	1	2	3	4	5
Fiber, g/d	13	16	19	23	29
RR	1.00	0.77	0.68	0.59	0.53
Adjusted RR	1.00	0.94	0.90	0.82	0.78

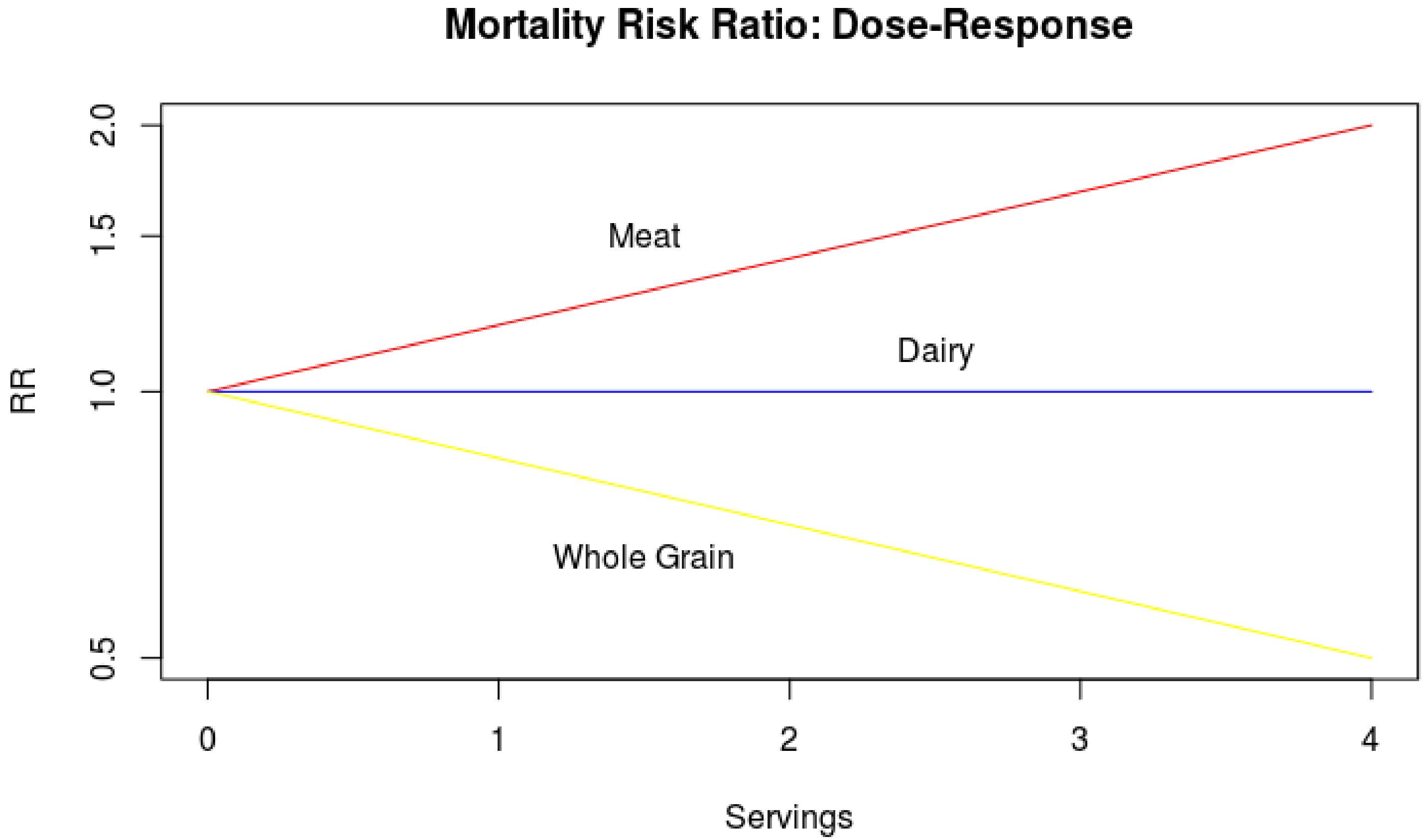
Multiple regression analysis

Estimates effect of main factor after taking effects of other co-factors into account

Fiber effect falls from 47% reduction in mortality to 22% after accounting for co-factors

Dose-response curve

Plotting mortality ratio vs. magnitude of the exposure

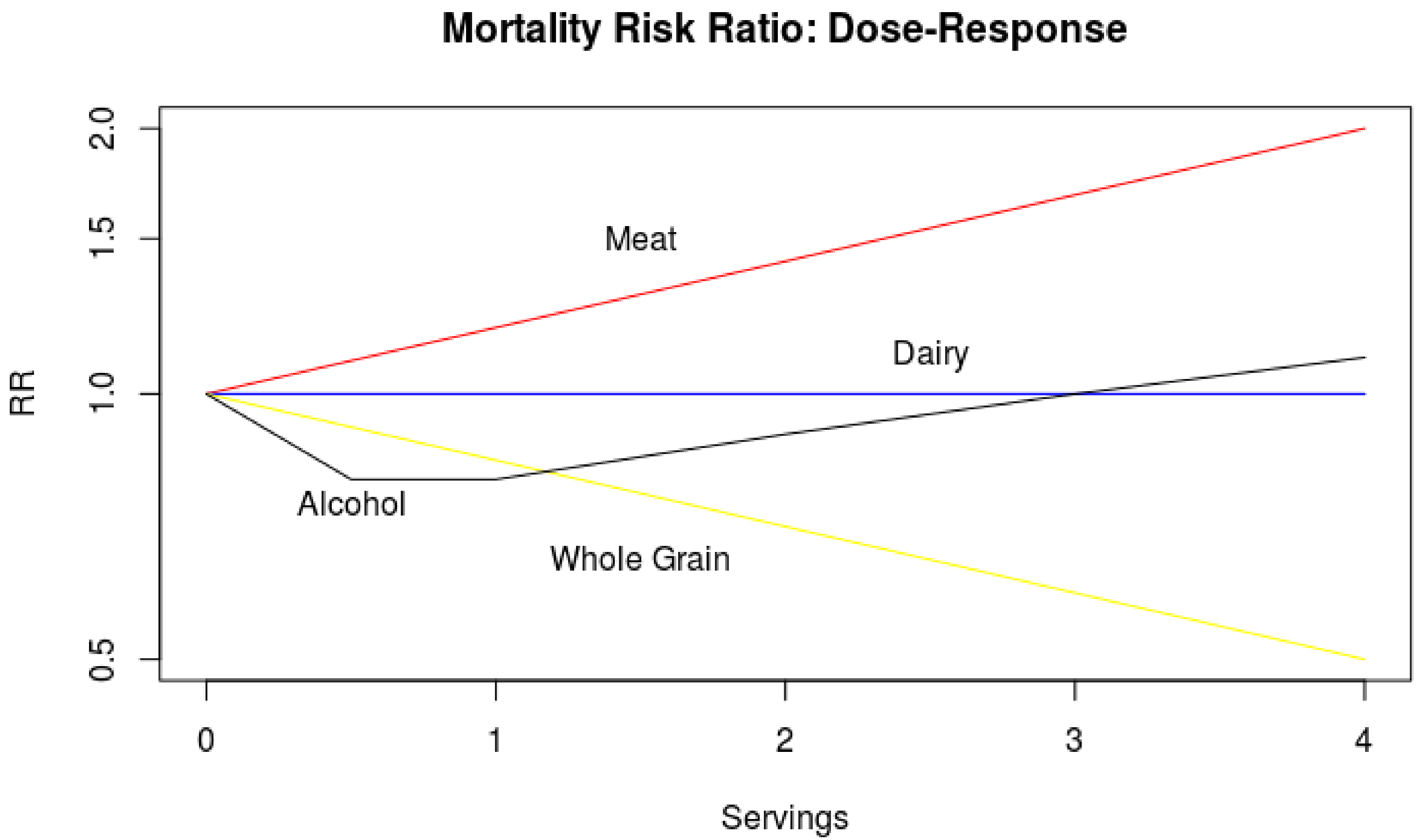


Dose-response curve

Rising D-R → higher risk of death; harmful

Falling D-R → lower risk of death; beneficial

U-shaped D-R → beneficial at low dose, but harm with increasing dose



Meta-analysis

A study of studies

Results from multiple similar studies are combined

Increases ability to detect small effects that can't be detected in individual studies

Often presented as forest plots

Special tests to check for biases that could invalidate results (publication bias, inhomogeneity)

Forest plots

Compact graphical depiction of RR and 95% CI

Combining data from multiple studies: Meta-analysis

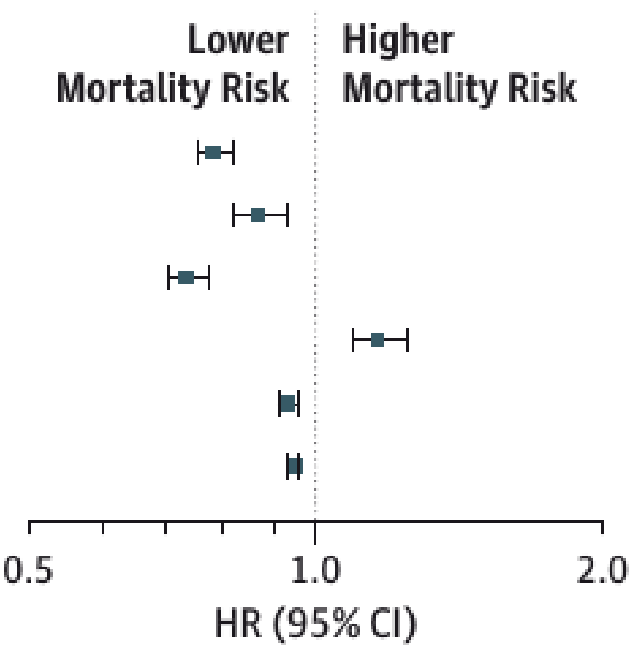
Comparing data from multiple factors

Icon size indicates relative number of subjects

Horizontal line spans CI, usually 95% CI

A Total mortality

	HR (95% CI)
UFAs, 5% of energy	0.78 (0.75-0.82)
MUFAs, 5% of energy	0.87 (0.82-0.93)
PUFAs, 5% of energy	0.73 (0.70-0.77)
Trans-fat, 2% of energy	1.16 (1.09-1.24)
ω-6 PUFAs, 2% of energy	0.93 (0.91-0.96)
ω-3 PUFAs, 0.3% of energy	0.95 (0.93-0.96)



Causation vs. correlation

Regression analysis finds *associations* (correlation) between outcome and risk factors

Correlation does not prove causation

However, causation becomes more tenable when:

- A biologically plausible mechanism exists to support a cause-effect linkage
- Multiple studies replicate relationship
- Reverse causation is excluded

Lack of correlation does not disprove causation

A negative study does not establish the lack of an effect of a factor

Accurate measurement of intake levels is a gnarly problem in dietary studies

- Total sugar intake especially inaccurate
- Bias in measurements related to gender, BMI, other factors

Mis-measurement drives relative risks toward the null (RR of 1.0, non-significant)

Biomarkers may be used to correct for bias and yield more accurate estimates of intake

- Urinary sugar excretion can be used to adjust for diet questionaire bias

More information

Web site for our class:

http://olli-what-to-eat-and-why.weebly.com

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