Where's the Beef?

The Ecstasy and the Agony of Devouring Flesh

Edwin Cox, M.D.

Fall 2019

Meat and Nutrition

Pros

- To a meat lover, there is no purer pleasure in life than a sizzling steak right off the grill
- Red meat provides high-quality protein, as well as creatine and iron for building strong muscles

Meat and Nutrition

Cons

- Greater resources consumed (soil, fertilizer, land) to produce equivalent nutrition vs. plants
- Possible adverse health consequences – CVD, diabetes, cancer
- And, guess what, ….

Red meat kills!

Red meat kills

Regular beef and/or pork consumption is a lethal habit

- Multiple studies agree: eating more red meat = higher mortality
- Unprocessed and processed meats (bacon, lunch meats, hot dogs, etc) both convey risk

Bottom line on meat

There is no nutritional requirement for meat

- Plant-sourced protein is fully adequate, as long as care is taken to get all essential amino acid

White meat (poultry) has no associated health threats that have been reported

- Except for rare bacterial contamination of raw product

Red meat (beef, pork) kills!

- Multiple high-quality studies are now in agreement, that excess mortality is highly associated with dietary red meat
- Processed meats (bacon, lunch meats, hot dogs, etc) are especially lethal

Why haven't we heard this?

If red meat is so dangerous, why haven't the health authorities and the media informed us?

1) The definitive data are hot off the press

- Published in 2017

2) Unclear why the media has missed it

- Too complicated, not sexy enough

3) Meat industry infiltrates USDA and other committees that generate guidelines

4) Some think it is "old news" → saturated fats

- WRONG!

What about low-carb, meat-dominant diets?

The Atkins diet and similar weight-control programs rely heavily on meat

This approach may be safe and effective as a short-term strategy to preserve muscle mass while shedding excess fat

But…

A long-term maintenance diet based on red meat is risky business, based on best current evidence

Early Work – Meat & Health

Ancel Keys

- Nutrition researcher for U.S. Army during WWII
- Development of K-rations, studies of starvation
- Recognized epidemic of coronary heart disease in middle-class Americans in 1950s, and associated it with diet
- Focused on high saturated fat content of meats and dairy products as likely culprit; recommended moderation in consuming these

The "fat is bad" hypothesis

The work of Keys and others was misinterpreted

A U.S. Senate Commission – the McGovern Commission – declared that people should limit consumption of all dietary fat

The idea that all fats contribute to CHD – and were its main cause - was widely promoted

Low-fat diets became the standard dietary recommendation from mid 1980s into the 2000s

- Food Pyramid created by the USDA banished fats to the top of the pyramid
- Grains were depicted as the foundation of the diet: 6-11 servings per day

Meat and fat

Meat was targeted for attention because of its high fat content

The public was advised that meat was fine, as long as lean cuts were selected and fat was trimmed

Carbs to the fore

Low-fat products were churned out by the food industry

- Prominently labeled "healthy" because they had little or no fat
- Fats were replaced with carbohydrates

However, an epidemic of obesity and diabetes followed

The carb skeptics

Dr. Robert Atkins was the vanguard in the "carbs are bad" revolution

The Atkins diet, with marked restriction of carb consumption, allowed liberal protein and fat

His followers did lose weight, at least for a while

- Placeholder: the ketogenic diet, not to be confused with the "Keto Diet", is an important development in addressing obesity; discussed in another session

Atkins diet went "viral"

Atkins was joined by Paleo and a host of others, basically claiming you could be fit and healthy, as long as you kicked carbs to the curb

No systematic trials to assess long-term effects on health; it was tacitly "accepted" that the weight loss was the "canary in the coal mine", proving its benefits

Sadly, they were wrong

Simply avoiding carbs and liberalizing animal protein and fat may have short-term benefits

However, long-term consumption of red meat is a losing proposition health-wise

It took large-scale, high-quality epidemiology studies to demonstrate the harms of red meat

- NIH-AARP
- NHS and HPFS
- EPIC

NIH-AARP Diet & Health Study

Mortality from different causes associated with meat, heme iron, nitrates, and nitrites in the NIH-AARP Diet and Health Study: population based cohort study

Arash Etemadi, Rashmi Sinha, Mary H Ward, Barry I Graubard, Maki Inoue-Choi, Sanford M Dawsey, Christian C Abnet

British Medical Journal, 2017

Meat & Mortality

NCI-AARP Diet & Health Study

- 1995 – Invitations sent to 3.5 million AARP members in six U.S. states
- 536,969 subjects (59% male, 41% female)
- Ages 50-71 at intake
- Predominantly white, more education than U.S. population, with fewer smokers, less fat and red meat, more fruits and vegetables consumed

NIH-AARP Study

Methods

- Prospective cohort – observational study
- FFQ 124 items, validated by 24 hr recall
- Meat intake categorized as total, processed, and unprocessed red meat
- White meat evaluated but will be presented separately
- 15 year follow-up with cause of death ascertainment
- Multivariate statistical analysis

NIH-AARP Meat and Mortality

Characteristics of NIH-AARP Diet and Health Study				
	Subjects	Deaths	Deaths CVD	Deaths Cancer
Males	316,505	84,848 (27%)		
Females	220,464	43,676 (20%)		
Total	536,969	128,524 (24%)	34,723	45,740

NIH-AARP Meat & Mortality

Table 1. All-cause Mortality vs. Daily Red Meat Consumption					
Quintile	1	2	3	4	5
Subjects	107,393	107,393	107,393	107,393	107,393
Red Meat consumed (oz/day)	0.6	1.5	2.2	3.1	4.7
Deaths expected	22,075	22,075	22,075	22,075	22,075
Deaths observed	22,075	23,765	25,532	27,321	29,831
Excess deaths (%)	0%	7.7%	16%	24%	35%

NIH-AAPR Meat and Mortality

Let's look at quintiles 2-5

88,300 deaths were expected (4 x 22,075)

106,449 death were observed

That was 18,149 (20.6%) more than expected

It was extremely unlikely due to chance

Therefore, red meat – or something highly associated with red meat – increased the death rate by 20%!

Guilt by association

Twins Jack and Jake go everywhere together

Whenever they come to my house for dinner, something goes missing afterward

- A piece of china, a silver serving piece, a CD, you name it

It becomes clear that the twins are responsible for the disappearances

Is Jack the klepto? Or is it Jake?

Or are they involved equally – one does the deed one time, another the next?

No way to resolve without catching them in the act – or having some independent assessment of which of them has sticky fingers

Guilt by association: statistics

Regression analysis demonstrates association between outcomes (e.g., death) and risk factors (e.g., eating meat)

If two factors (A & B) are correlated and each is associated with the outcome, statistical tests cannot resolve among these possibilities:

- A is causative and B is merely guilty by association
- Vice versa
- Both contribute to the outcome

In the end, the regression analysis does the best it can: assign responsibility to both in some proportion

The researcher must try to find other evidence to establish the chain of causation

Risk factors: NIH-AARP

If the increased mortality was not due to eating red meat, what was that "something else" associated with eating red meat?

Those eating more red meat:

- Higher rate of cigarette smoking
- Lower rate of physical activity

Risk factor analysis needed to establish that other risk factors were not responsible for meat's apparent lethality

NIH-AARP Meat and Mortality

Relative Risk of mortality per 20 g per 1,000 kcal per day increase in meat			
	All-cause	CVD	Cancer
Total red meat	1.09	1.08	1.07
Unprocessed red meat	1.08	1.08	1.06
Processed red meat	1.10	1.08	1.08

$P < 0.001$

Subjects with disease at baseline excluded

20 g per 1,000 Cal is 45 g (1.5 oz) per day for average size man

RR (all-cause, total red meat) for 3 oz is 1.09 x 1.09 = 1.19 (19% increase)

Covariates accounted for: age, smoking, physical activity, education, marital status, family history (cancer), race, BMI, alcohol intake, vitamin use, hormone therapy

NIH-AARP Study

Results

- Increasing all-cause mortality, CVD mortality, cancer mortality, and other mortality with increasing consumption of total red meat, unprocessed red meat and processed meat
- Relationship persisted after accounting for effects of other variables

NIH-AARP

Conclusions

- Total amount of red meat positively associated with increased mortality
- Amount of unprocessed red meat positively associated with increased mortality
- Amount of processed red meats positively associated with increased mortality

NHS - HPFS

Red Meat Consumption and Mortality: Results from Two Prospective Cohort Studies

An Pan, PhD, **Qi Sun, MD, ScD**, **Adam M. Bernstein, MD, ScD**, **Matthias B. Schulze, DrPH**, **JoAnn E. Manson, MD, DrPH**, **Meir J. Stampfer, MD, DrPH**, **Walter C. Willett, MD, DrPH**, and **Frank B. Hu, MD, PhD**

Departments of Nutrition (Drs Pan, Sun, Bernstein, Stampfer, Willett, and Hu) and Epidemiology (Drs Manson, Stampfer, Willett, and Hu), Harvard School of Public Health, Boston, Massachusetts; Channing Laboratory (Drs Sun, Stampfer, Willett, and Hu) and Division of Preventive Medicine (Dr Manson), Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; Wellness Institute of the Cleveland Clinic (Dr Bernstein), Lyndhurst, Ohio; Department of Molecular Epidemiology (Dr Schulze), German Institute of Human Nutrition, Arthur-Scheunert-Allee 114-116 Nuthetal 14558, Germany

Archives of Internal Medicine, 2013

Meat & Mortality

Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)

- 83,644 women & 37,698 men
- Studies begun in the 1980s, now with follow-up data approaching 30 years
- Homogeneous, well-educated populations with high rate of cooperation

NHS - HPFS

Methods

- Prospective cohort – observational
- FFQ 131-166 items
- Covariates: age, BMI, race, smoking, alcohol use, physical activity, multivitamin use, aspirin use, family history (diabetes, CVD), personal history (diabetes, hypertension, hypercholesterolemia)
- Follow-up: Up to 28 years
- Multivariate statistical analysis
- Serving
  - Unprocessed – 3 oz (85 g)
  - Processed – 15 g (bacon), 28 g (sandwich meat), 45 g (hot dog)

NHS-HPFS Meat and Mortality

Study	#	Age Range	Total deaths	CVD deaths	Cancer deaths
NHS	83,644	30-55	15,000	3,194	6,391
HPFS	37,698	40-74	8,926	2,716	3,073

NHS-HPFS Meat and Mortality

Red meat intake and RR of all-cause mortality (HPFS)						
		Q1	Q2	Q3	Q4	Q5
Total red meat	Servings/d	0.25	0.61	0.95	1.36	2.07
	RR	1.0	1.12	1.21	1.25	1.37
Unprocessed red meat	Servings/d	0.17	0.43	0.66	0.95	1.46
	RR	1.0	1.11	1.14	1.20	1.29
Processed red meat	Servings/d	0.02	0.13	0.21	0.39	0.74
	RR	1.0	1.06	1.15	1.18	1.27

NHS-HPFS Meat and Mortality

Relative Risk per 1 serving / day increase in meat (NHS/HPFS)			
	All-Cause	CVD	Cancer
Total red meat	1.12	1.16	1.10
Unprocessed red meat	1.13	1.18	1.10
Processed red meat	1.20	1.21	1.16

$P < 0.001$

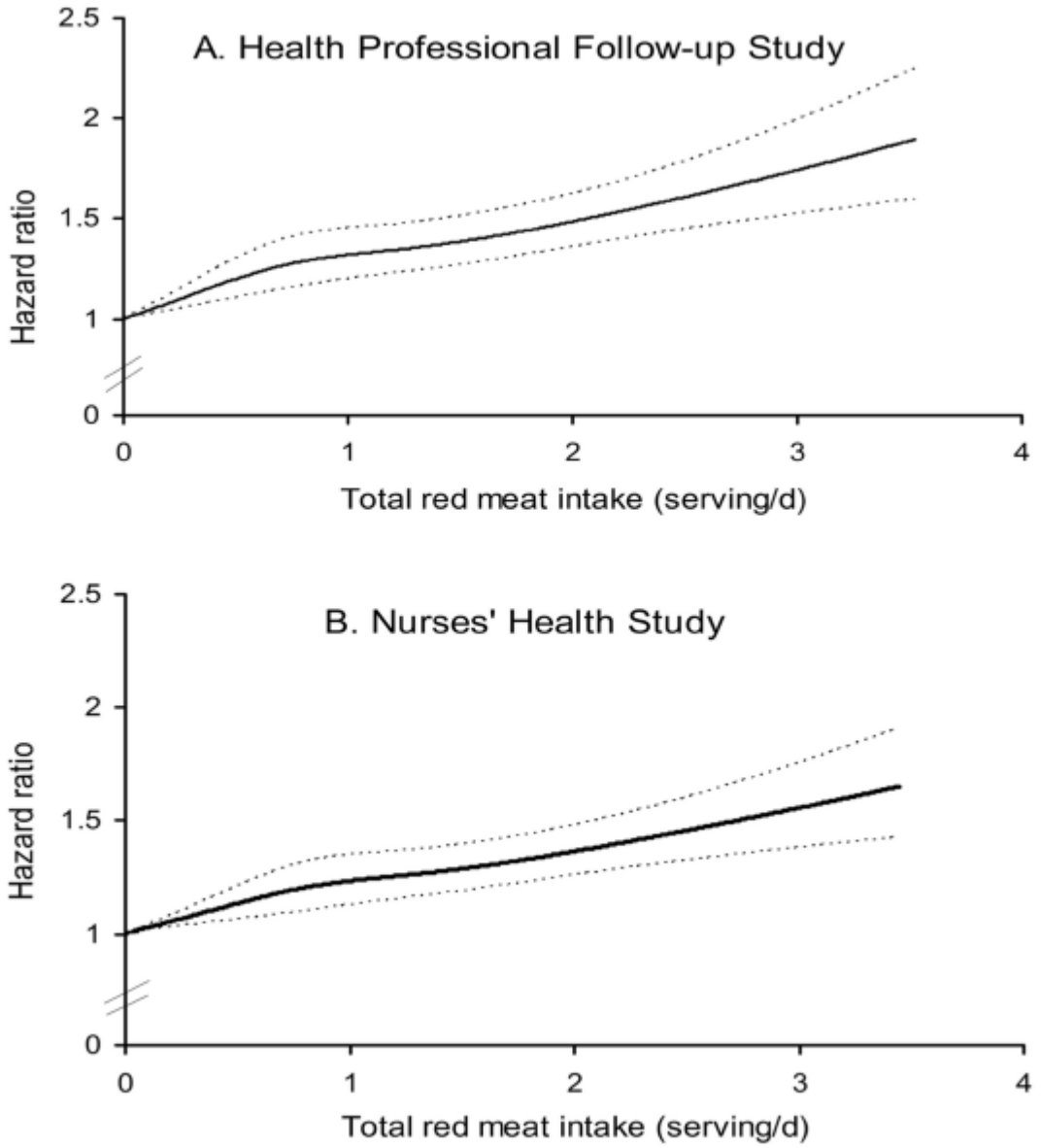


Figure 1. Dose-response relationship between red meat intake and risk of all-cause mortality in (A) Health Professionals Follow-up Study and (B) Nurses' Health Study

Red Meat & Mortality

Study	Type	Subjects	Events	Follow-up
NIH-AARP	Observational	536,000	24%	15 yr
NHS-HPFS	Observational	121,000	20%	20+ yr

Red Meat & Mortality

Study	Processed Red Meat	Unprocessed Red Meat	Total Red Meat
NIH-AARP	21%	17%	19%
NHS-HPFS	20%	13%	12%

Increased RR of mortality per 100 gm of red meat per day

The latest…

Annals of Internal Medicine REVIEW

Red and Processed Meat Consumption and Risk for All-Cause Mortality and Cardiometabolic Outcomes

A Systematic Review and Meta-analysis of Cohort Studies

Dena Zeraatkar, MSc; Mi Ah Han, MD, PhD; Gordon H. Guyatt, MD, MSc; Robin W.M. Vernooij, PhD; Regina El Dib, PhD; Kevin Cheung, MD, MSc; Kirolos Milio, BSc; Max Zworth, BASc; Jessica J. Bartoszko, HBSc; Claudia Valli, MSc; Montserrat Rabassa, PhD; Yung Lee, BHSc; Joanna Zajac, PhD; Anna Prokop-Dorner, PhD; Calvin Lo, BHSc; Malgorzata M. Bala, PhD; Pablo Alonso-Coello, MD, PhD; Steven E. Hanna, PhD; and Bradley C. Johnston, PhD

Oct 1, 2019 NutriRECS

All-cause mortality and red meat

Meta-analysis: 8 studies; 903,000 subjects

Follow-up: 10.8 yrs (avg)

Deaths: 11.3%

RR: 0.88 (0.84-0.93) for reduction of 3 servings per week

"14 fewer deaths per 1,000 subjects over the 10.8 years of followup"

All-cause mortality and red meat

The flip-side:

Everyone will die – 100%

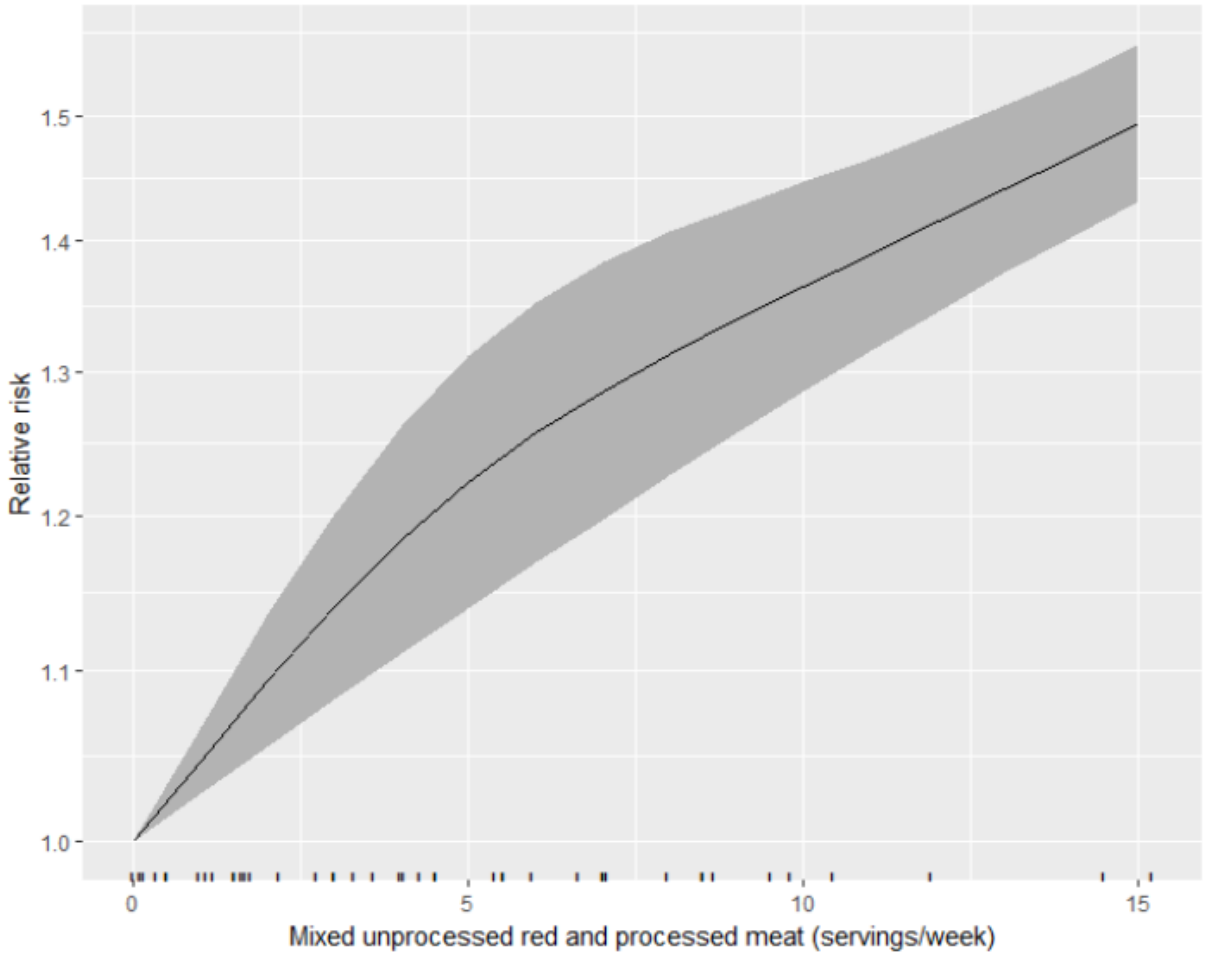
The exposure is lifetime, so the risk should extend over the lifetime

No evidence was provided that the relative contribution of meat to mortality is age-dependent

Eventual number of deaths due to meat at 3 servings per week would be estimated as 14 x (100/11.3) = 124 per thousand

However, Americans eat twice that much meat. Estimated contribution of meat to American mortality could be 250 out of every thousand deaths!

All-cause mortality and red meat



NutriRECS 2019

NutriRECS Conclusion

Conclusion: The magnitude of association between red and processed meat consumption and all-cause mortality and adverse cardiometabolic outcomes is very small, and the evidence is of low certainty.

What the ????

Very small?

- Because they focused only on the years of exposure; didn't consider lifetime

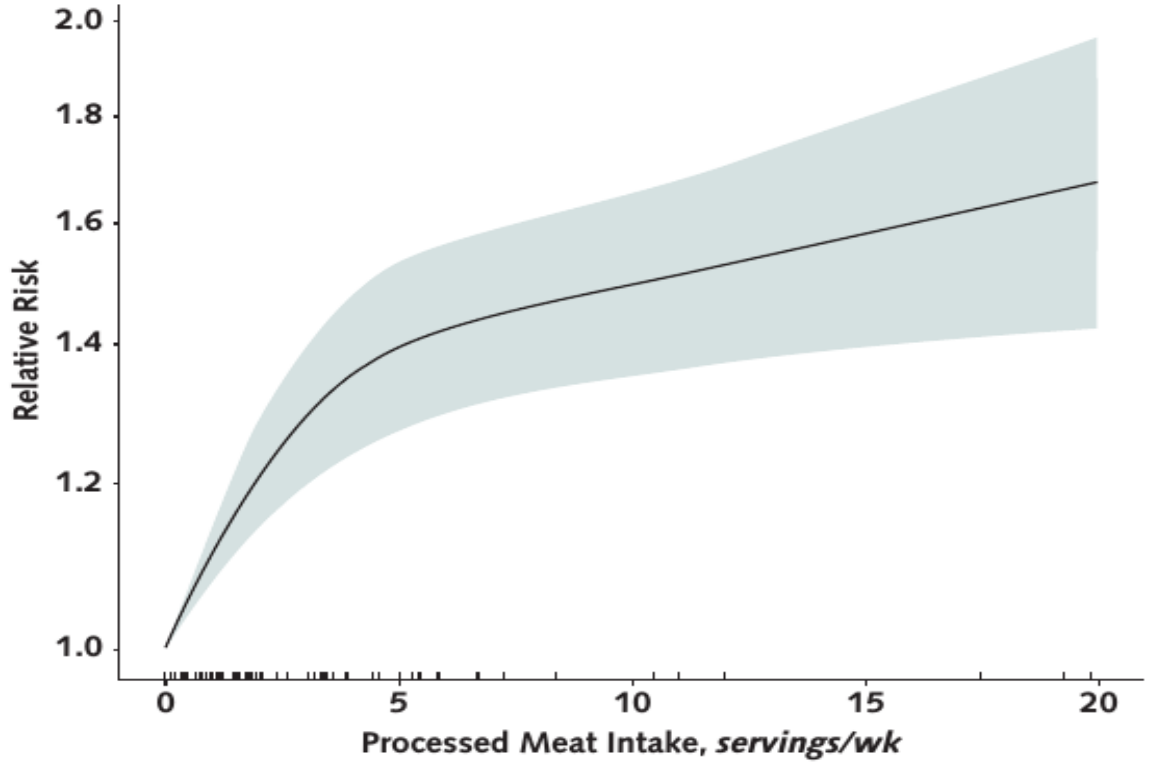
Evidence of low certainty?

- Measurement error
- Residual confounding – Was is Jack or Jake?

My take: They have gone off the rails!

Diabetes and red meat

Figure. Nonlinear association between processed meat intake and type 2 diabetes.



NutriRECS 2019

White meat and mortality?

NHS-HPFS

- Substitution analysis – significant RR when compared with red meat

NIH-AARP

- Next slide

White meat and mortality

Relative Risk of mortality per 20 g per 1,000 kcal per day increase in meat			
	All-cause	CVD	Cancer
Total white meat	0.92	0.92	0.93
Unprocessed white meat	0.92	0.92	0.94
Processed white meat	0.95	0.96*	0.92

$P < 0.001$, except * NS

NIH-AARP Meat and Mortality Study

Processed Meats and Risk

What are processed meats?

- Bacon, sausage, deli meats, hot dogs
- Red meat (skeletal muscle) plus offal (organs, entrails)

What components are otherwise different between unprocessed and processed meats

→ next slide

Table 2. Differences in Average Nutritional and Preservative Contents Between Red Meats and Processed Meats per 50-g Servings, as Consumed in the United States

Per 50 g of Meat	Red Meats, Mean±SE (Median)	Processed Meats, Mean±SE (Median)
Energy, kcal	123.3±0.7 (124.1)	138.1±2.0 (150.6)
Total fat, % energy	49.6±0.3 (54.1)	57.5±0.6 (69.4)
Total fat, g	7.1±0.1 (7.7)	10.2±0.2 (12.3)
Saturated fat, % energy	18.7±0.1 (20.4)	19.4±0.3 (22.8)
Saturated fat, g	2.7±0.0 (2.9)	3.5±0.1 (4.4)
Monounsaturated fat, % energy	21.4±0.1 (23.9)	25.3±0.3 (30.7)
Monounsaturated fat, g	3.1±0.0 (3.3)	4.5±0.1 (5.3)
Polyunsaturated fat, % energy	2.7±0.0 (1.7)	6.4±0.1 (6.1)
Polyunsaturated fat, g	0.4±0.0 (0.2)	1.1±0.0 (0.6)
Protein, % energy	46.2±0.3 (41.5)	35.4±0.5 (27.4)
Protein, g	13.6±0.0 (13.5)	9.8±0.1 (8.8)
Sodium, mg	154.8±3.4 (127.1)	621.7±7.6 (575.8)
Potassium, mg	161.0±0.8 (152.8)	170.2±1.9 (153.6)
Cholesterol, mg	41.9±0.2 (43.8)	34.1±0.3 (28.3)
Iron, mg	1.1±0.0 (1.2)	0.6±0.0 (0.6)
Nitrates, mg	3.3±0.0 (2.9)	4.6±0.1 (3.0)
Nitrites, mg	0.5±0.0 (0.7)	0.8±0.0 (0.6)
Nitrosamines, μg	0.1±0.0 (0.2)	0.3±0.0 (0.2)

Based on data from the 2005–2006 US NHANES and a report of published nitrate, nitrite, and nitrosamine contents of foods,[26] each analyzed according to actual US consumption levels and accounting for the NHANES sampling and weighting strategies. All mean differences were significant at the 0.05 level.

Substitution analysis

Substitute a serving of food B (for example, fish) for consumed food A (for example, red meat)

Do a statistical simulation based on observed risk factor analysis

Calculate the increase or reduction in risk from the substitution

Based on the NHS/HPFS data

NHS-HPFS Meat and Mortality

Relative Risk of replacing 1 serving per day of meat with designated food						
	Nuts	Poultry	Whole Grains	Dairy	Legumes	Fish
Total red meat	0.81	0.86	0.86	0.90	0.90	0.93
Unprocessed red meat	0.82	0.87	0.87	0.91	0.92	0.95
Processed red meat	0.78	0.83	0.84	0.87	0.87	0.90

$P < 0.001$

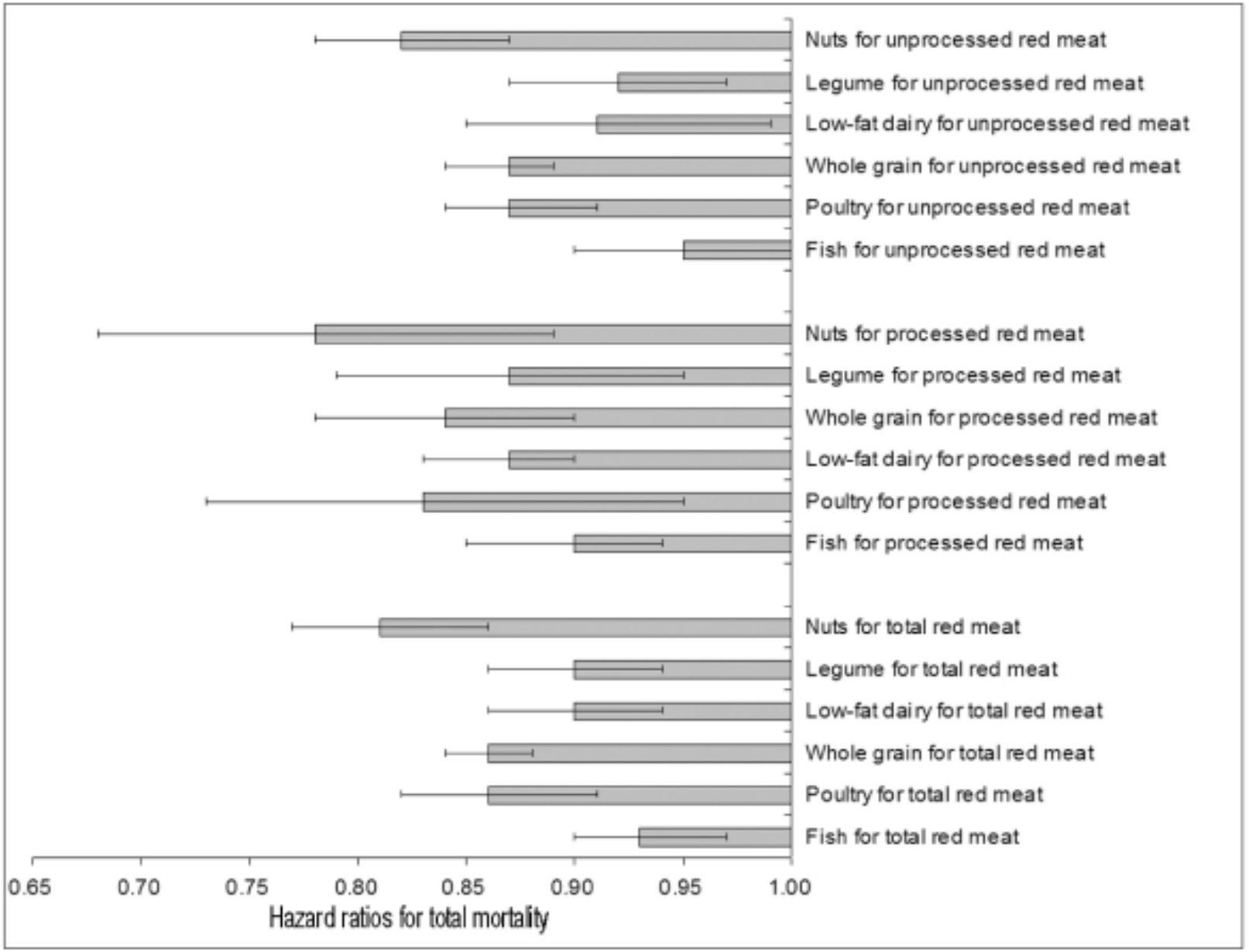


Figure 2. Hazard ratios and 95% confidence intervals for total mortality associated with replacement of other food groups for red meat intake

Meat substitute simulation

Eating most anything other than red meat (processed or unprocessed) would lower the total mortality rate

- Nuts by 20%, poultry and whole grains by 15%, beans and low-fat dairy by 10%, fish by 5%

Not all "apples-to-apples", because the ratio and quality of proteins not equivalent between foods

- However, poultry vs. red meat comparison should be appropriate

Why would red meat cause heart disease?

Classical mechanisms proposed for CVD effects

- Saturated fat and/or cholesterol content
- Heme iron
- Nitrites (processed)
- Sodium (processed)

Data not very convincing; other factors suspected – stay tuned

Why would red meat cause cancer?

Classical mechanisms proposed for cancer related to red meat

- Carcinogenic compounds produced by high temperature cooking
  - Nitrosamines / nitrosamides
  - Polycyclic aromatic hydrocarbons
  - Heterocyclic amines
- Heme iron / iron overload
  - N-nitroso compound formation
  - Cytotoxicity / epithelial proliferation
  - Oxidative stress / hypoxia signaling
- Saturated fat (breast, colorectal cancer)

As with CVD, not very convincing

Red meat and disease: ***Breaking news***

Excess dietary protein, especially containing branched-chain amino acids, is associated with shortened lifespan and increasing rates of cancer, cardiovascular disease and diabetes

Choline-associated compounds – prominent in red meat – are converted into an ASCVD-promoting compound, TMAO, via the gut microbiome

Dietary protein, lifespan and healthspan

The lifespan – and healthspan – of many different species can be increased by calorie restriction (CR) with optimal nutrition, i.e., sufficient vitamins and other micronutrients

Recent work pinpoints protein restriction (PR) as the most specific effective dietary intervention

- PR without CR increased the lifespan and health of mice in a systemic investigation across a matrix of different proportions of carbohydrate, fat, protein and total energy
- Restriction of specific amino acids pointed to branched-chain amino acids as most effective in prolonging life and reducing cancer and glucose intolerance

Mutations that affect aging

Ames dwarf mouse

- Spontaneous mutation described 1961
- Absent growth hormone, thyroid stimulating hormone, prolactin secretion
- 45-70% increase in lifespan
- Reduced IGF-1
- Reduced glucose and insulin
- Reduced reactive oxidative species

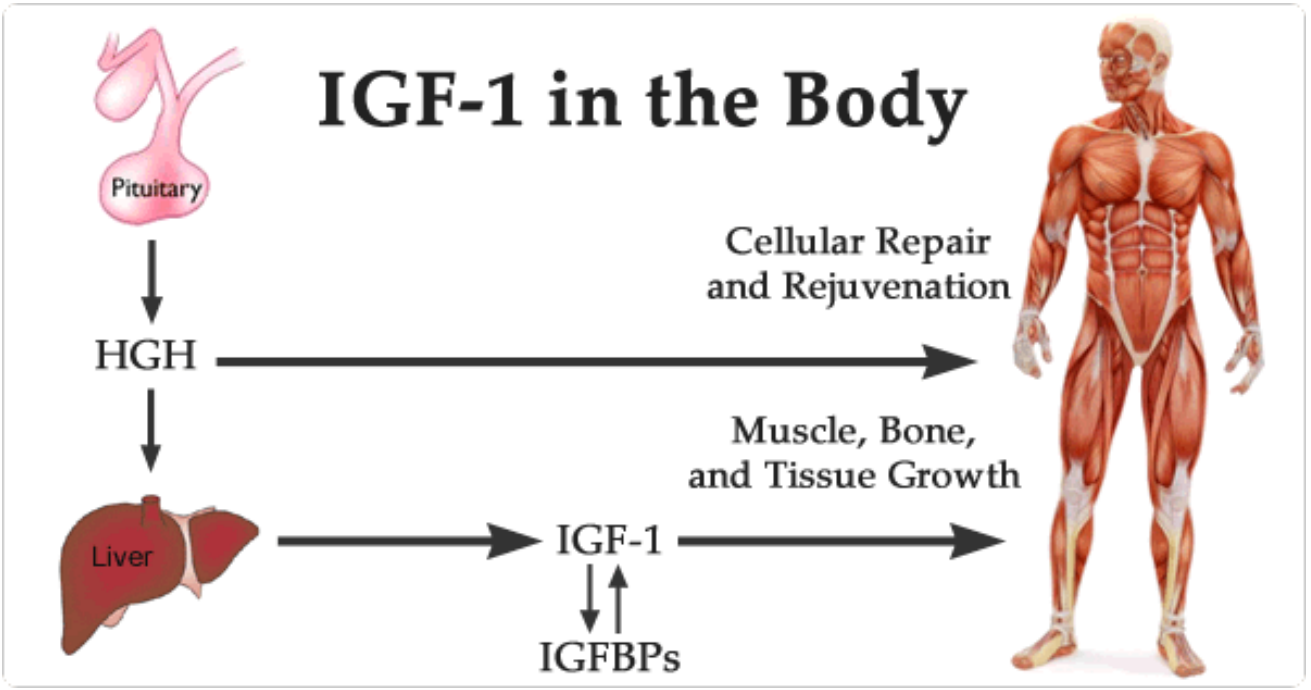
GH and IGF-1

Growth hormone (GH) is released by the pituitary and stimulates IGF-1 (insulin-like growth factor) production in the liver

IGF-1 stimulates proliferation of bones, muscles and other organs and tissues

Failure in GH and/or IGF-1 lead to short stature / dwarfism

Mutations in mice affecting GH (deficiency) and IGF-1 signaling are associated with longer life and striking reduction in cancer



Pathways to delayed aging

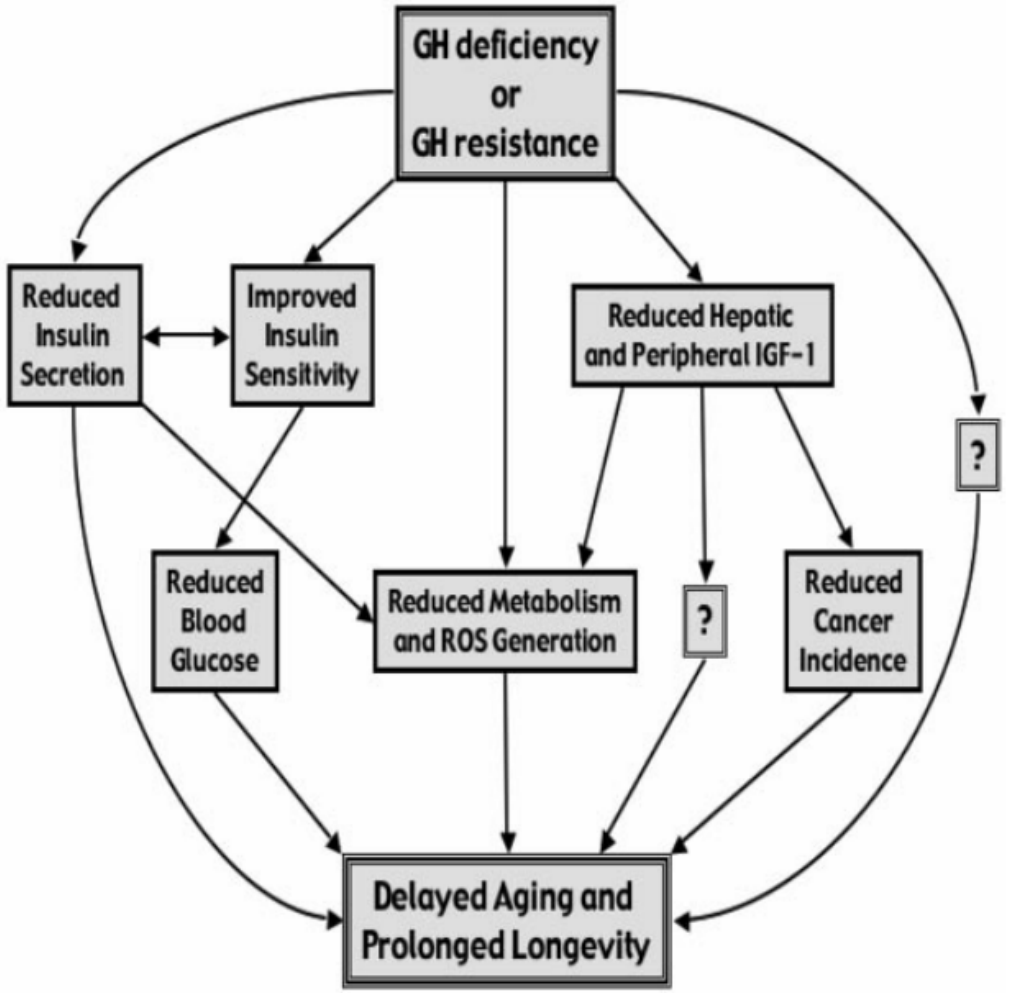
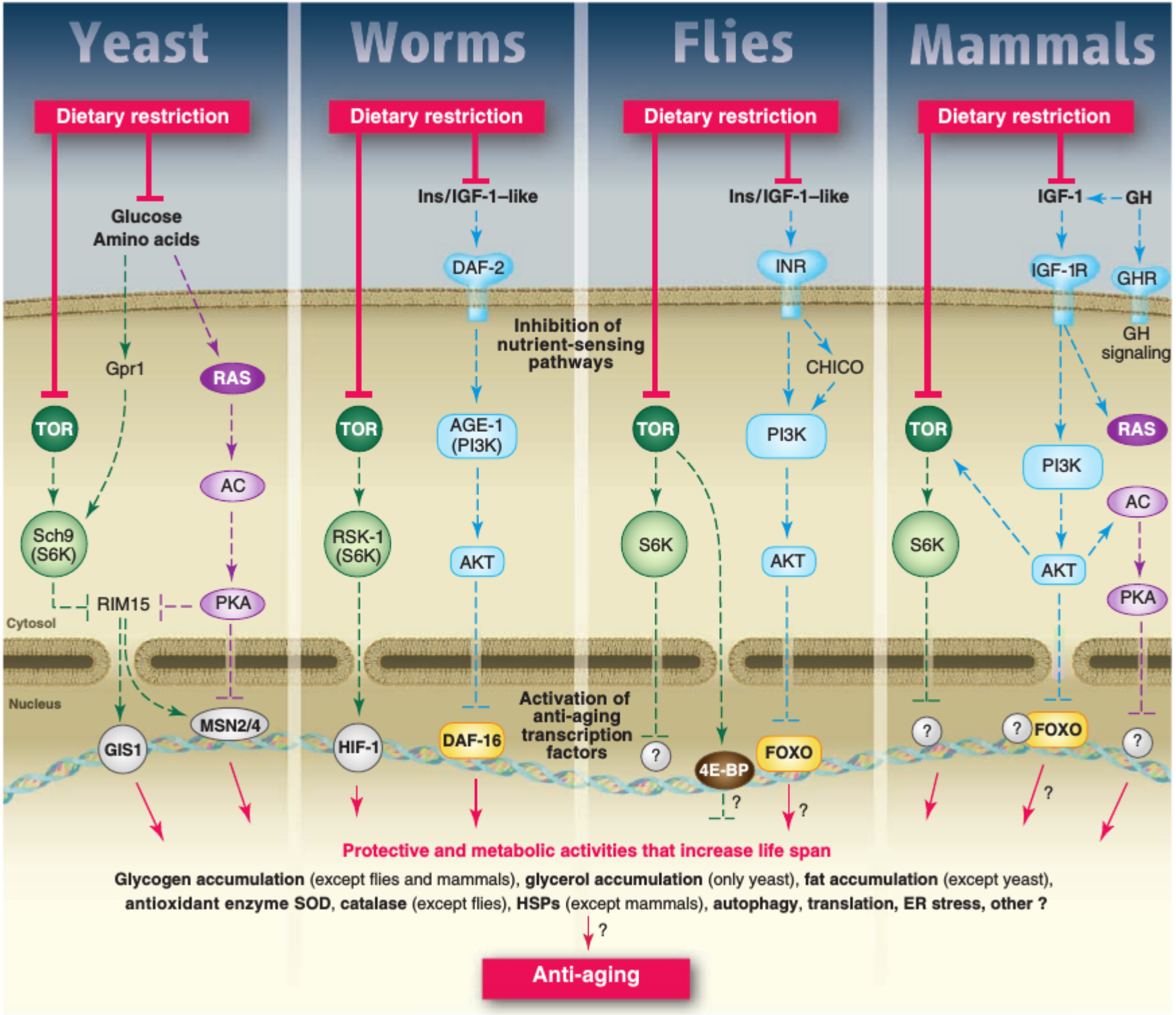


FIG. 1. Proposed mechanisms of prolonged longevity of hypopituitary, GH-deficient, and GH-resistant mice.


Yeast
Worms
Flies
Mammals
Dietary restriction
Glucose
Amino acids
Gpr1
RAS
TOR
AC
Sch9 (S6K)
RIM15
PKA
Cytosol
Nucleus
MSN2/4
GIS1
Ins/IGF-1–like
DAF-2
Inhibition of nutrient-sensing pathways
AGE-1 (PI3K)
AKT
RSK-1 (S6K)
HIF-1
DAF-16
Activation of anti-aging transcription factors
INR
CHICO
PI3K
S6K
4E-BP
FOXO
IGF-1
GH
IGF-1R
GHR
GH signaling
?
Protective and metabolic activities that increase life span
Glycogen accumulation (except flies and mammals), glycerol accumulation (only yeast), fat accumulation (except yeast),
antioxidant enzyme SOD, catalase (except flies), HSPs (except mammals), autophagy, translation, ER stress, other ?
Anti-aging

NHANES III study

Low Protein Intake is Associated with a Major Reduction in IGF-1, Cancer, and Overall Mortality in the 65 and Younger but Not Older Population

Morgan E. Levine[a,1], **Jorge A. Suarez**[a,b,1], **Sebastian Brandhorst**[a,b], **Priya Balasubramanian**[a,b], **Chia-Wei Cheng**[a,b], **Federica Madia**[a,h], **Luigi Fontana**[c,d,e], **Mario G. Mirisola**[a,b,i], **Jaime Guevara-Aguirre**[j], **Junxiang Wan**[a,b], **Giuseppe Passarino**[f], **Brian K. Kennedy**[g], **Pinchas Cohen**[a,b], **Eileen M. Crimmins**[a], and **Valter D. Longo**[a,b,2]

National Health and Nutrition Examination Study

Cell Metabolism 2014

NHANES III study

6,381 subjects aged 50+ U.S. citizens, representative sample

- 3,039 age 50-65
- 3,342 age 66+
- 55% female

Followup: 18 years (intake 1988-94)

Outcomes: Mortality – all-cause (40%), cardiovascular (19%), cancer (10%), diabetes (1%)

Protein consumption: Low (<10% of calories) – 437, medium (10-19%) - 4,798, high (20%+) - 1,146

NHANES: Mortality vs protein

Relative Risk of All-Cause Mortality vs. Dietary Protein as Percent of Calories			
Age Protein →	Low (<10%)	Medium (10-19%)	High (20%+)
50-65 yrs	1.0 (ref)	1.34*	1.74
65+ yrs	1.0 (ref)	0.79	0.72

All-cause mortality increased with increasing dietary protein in middle age but decreased in older subjects as protein intake increased

Statistical significance: All $p<0.05$ except * (not significant)

NHANES: Mortality vs protein

Relative Risk of **Cancer** Mortality vs. Dietary Protein as Percent of Calories			
Age Protein →	Low (<10%)	Medium (10-19%)	High (20%+)
50-65 yrs	1.0 (ref)	3.06	4.33
65+ yrs	1.0 (ref)	0.67*	0.40

Cancer mortality increased with increasing dietary protein in middle age but decreased in older subjects as protein intake increased

Statistical significance: All $p<0.05$ except * (not significant)

NHANES: Mortality vs protein

Relative Risk of **Cardiovascular** Mortality vs. Dietary Protein as Percent of Calories			
Age Protein →	Low (<10%)	Medium (10-19%)	High (20%+)
50-65 yrs	1.0 (ref)	0.79*	1.03*
65+ yrs	1.0 (ref)	0.80*	0.78*

Cardiovascular mortality did not vary with amount of dietary protein for either middle aged adults or older adults

Statistical significance: All * (not significant)

NHANES III study: Conclusion 1

"We propose that up to age 65 and possibly 75, depending on health status, 0.7 to 0.8 grams of proteins/kg of body weight/day ... should be recommended instead of the 1–1.3 g grams of proteins/kg of body weight/day consumed by adults ages 19–70"

NHANES III study: Conclusion 2

"We also propose that at older ages, it may be important to **avoid** low protein intake and gradually adopt a moderate to high protein possibly mostly plant based consumption to allow the maintenance of a healthy weight and protection from frailty"

The TMAO story

Trimethylamine-N-oxide is a metabolite that is normally present in low concentrations in humans

High levels of TMAO are associated with markedly increased rates of ASCVD

TMAO is only present in high levels from dietary sources

The most frequent dietary source is indirect

- Choline-associated compounds (lecithin, carnitine) are converted to an intermediate – TMA – by gut bacteria
- TMA is absorbed and converted to TMAO by a liver enzyme FMO3

TMAO plays havoc with cholesterol transport and foam cells in arterial walls, leading to AS

Atherosclerotic cardiovascular disease (ASCVD)

Risk factors identified for atherosclerosis

- Lipoprotein ("cholesterol") metabolism – higher LDL and triglycerides, lower HDL
- Hypertension
- Obesity
- Metabolic syndrome → diabetes

Yet, much of the disease risk remained unaccounted for by these factors

- Who's the perpetrator?
- It makes a great detective story!

The detective – Stan Hazen

Cleveland Clinic is a major heart disease referral center

- Project GeneBank started around 2000, aiming to advance knowledge of the causes, prevention and treatment of cardiovascular disease
- Goal of enrolling 10,000 subjects
- Blood samples obtained for studies

Stanley Hazen, M.D., Ph.D.

- Proposed looking for molecules in blood associated with ASCVD

Hazen's mission: Track down the perp

Identify molecules associated with ASCVD

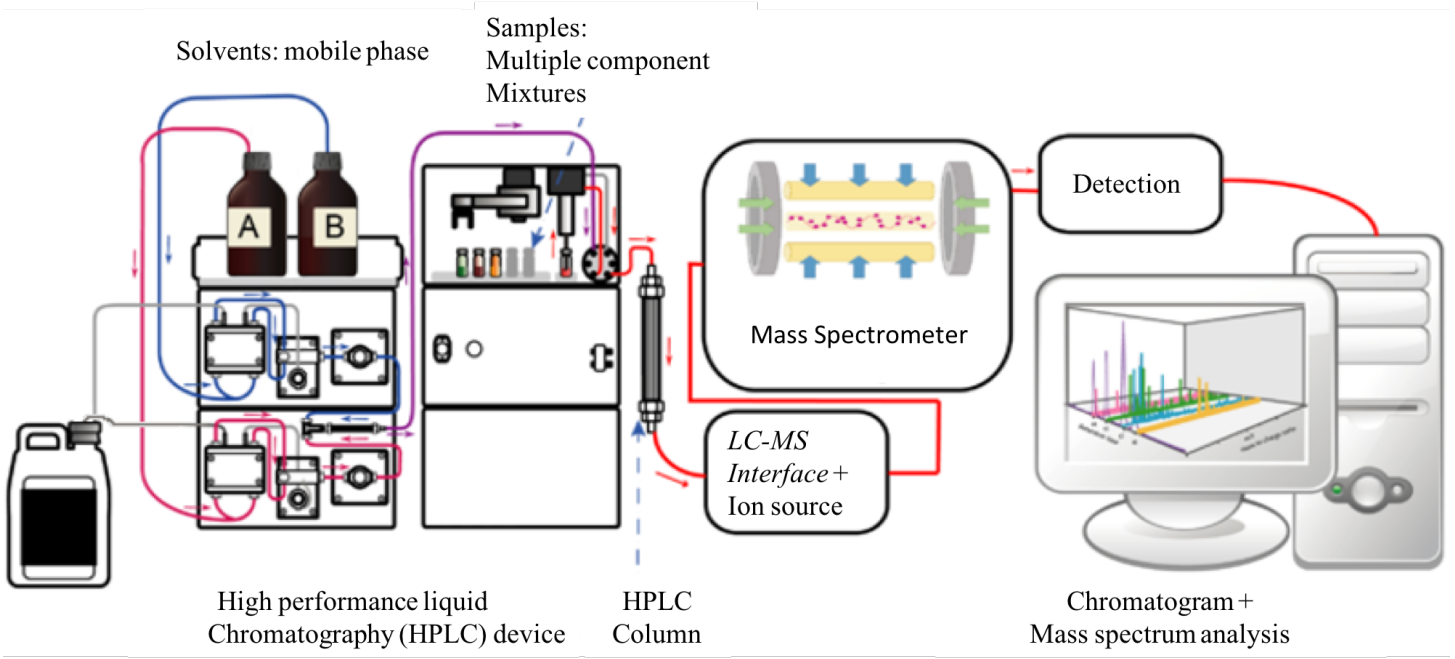
Finding the needle in the haystack

- Blood contains some 2,000 non-protein analytes (molecules)

Detective's high-tech tools

Liquid chromatography / mass spectrometry (LC/MS)

- Analytes separate in LC column by speed with which they flow in a solvent
- Each band of identical molecules characterized in MS by mass-to-charge ratio (m/z) → molecular weight & tentative ID



Detective's high-tech tools (cont'd)

Corfirm identity of molecules-of-interest by additional chemical analysis

Compare concentration of each between cases (patients with ASCVD) and normal controls

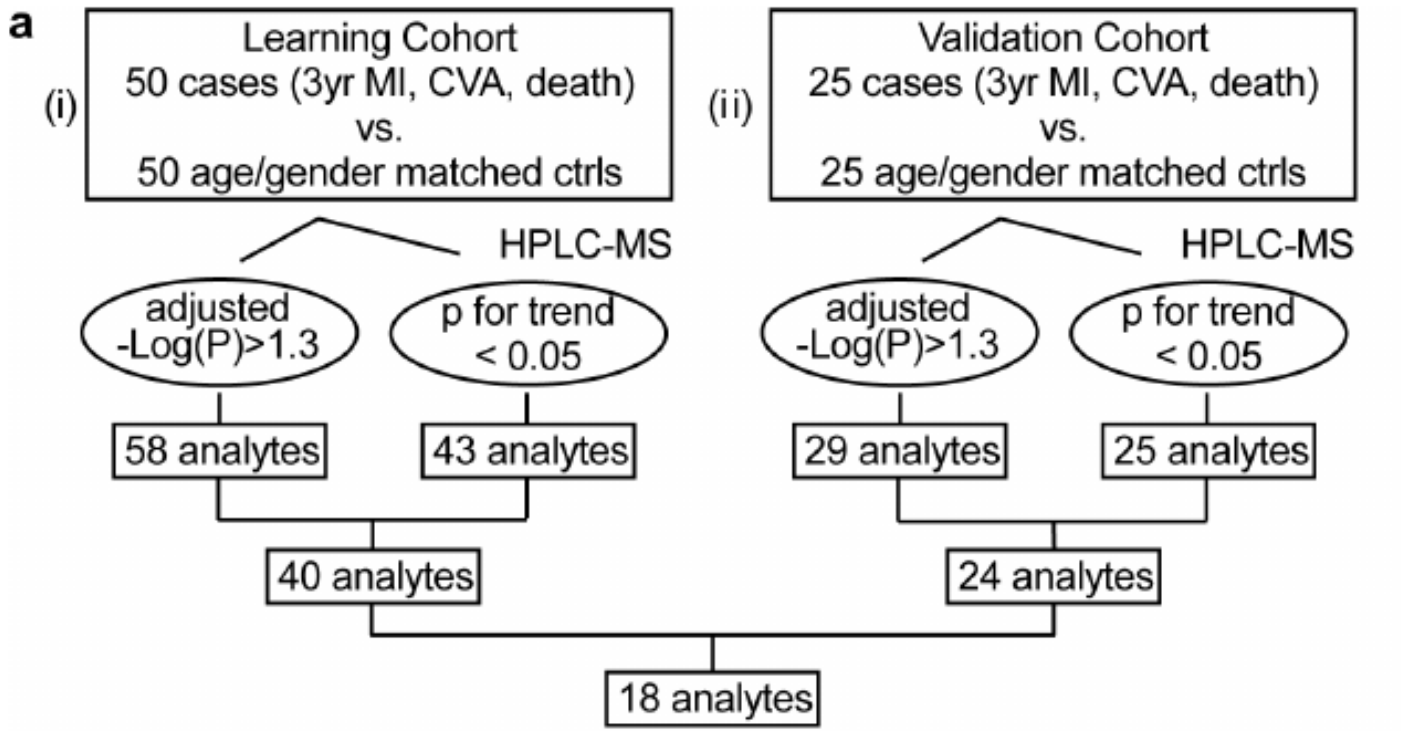
Focus in on compounds with significantly different concentrations

Nature 2011

Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Zeneng Wang[1,2], **Elizabeth Klipfell**[1,2], **Brian J. Bennett**[3], **Robert Koeth**[1], **Bruce S. Levison**[1,2], **Brandon DuGar**[1], **Ariel E. Feldstein**[1,2], **Earl B. Britt**[1,2], **Xiaoming Fu**[1,2], **Yoon-Mi Chung**[1,2], **Yuping Wu**[4], **Phil Schauer**[5], **Jonathan D. Smith**[1,6], **Hooman Allayee**[7], **W. H. Wilson Tang**[1,2,6], **Joseph A. DiDonato**[1,2], **Aldons J. Lusis**[3], and **Stanley L. Hazen**[1,2,6,8]

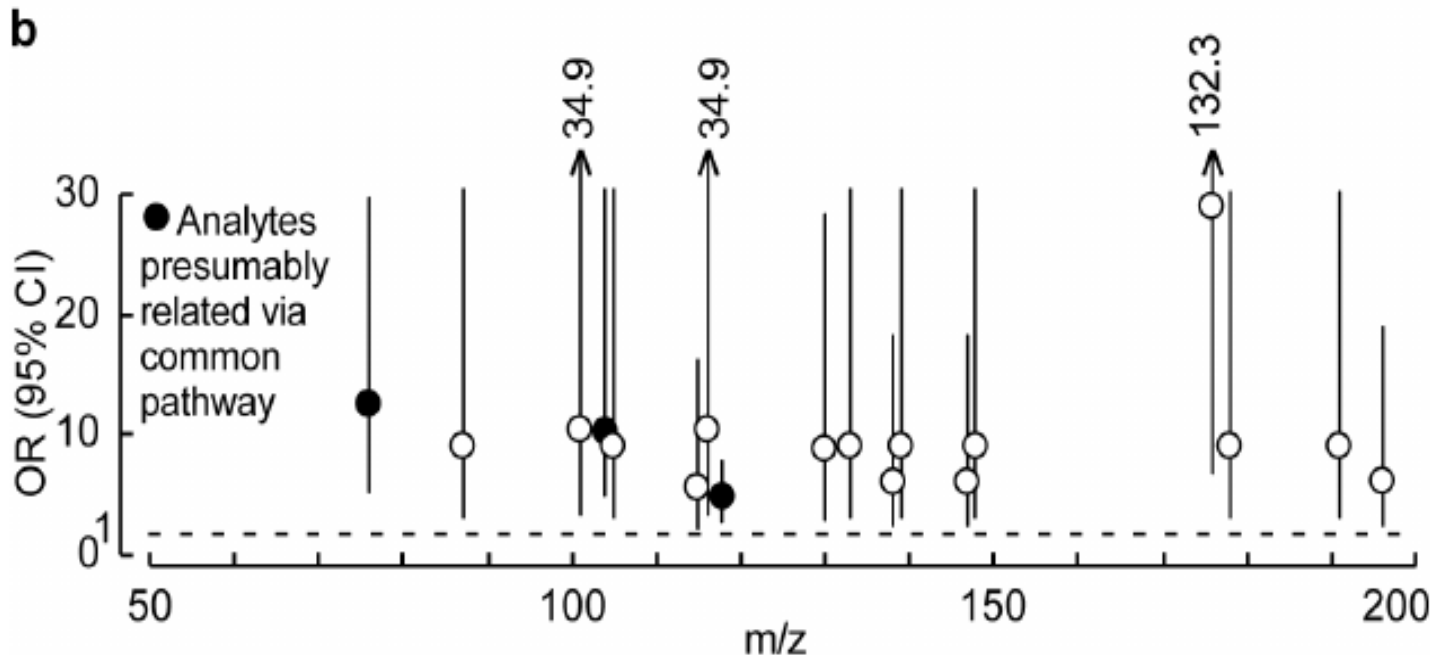
Identification strategy



(iii) Structural identification of analytes

(iv) Confirm clinical prognostic utility in independent prospective cohort (N=1876)

LC/MS analytes associated with higher ASCVD



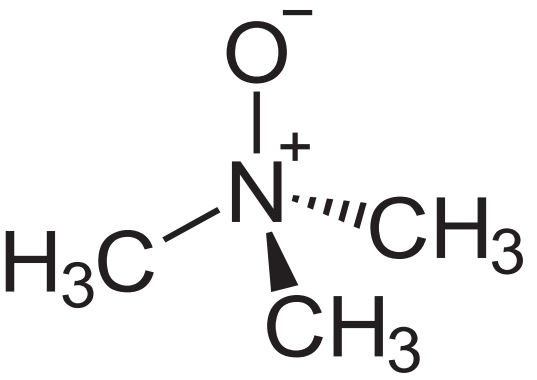
Three compounds that were associated with ASCVD were highly correlated among themselves; m/z 76, 104 and 118

Trimethylamine N-oxide (TMAO)

M/z 76 compound unequivocally ID'd as TMAO

TMAO is not in the typical human diet and plays no normal role in human metabolism

- What the heck is it doing there ??



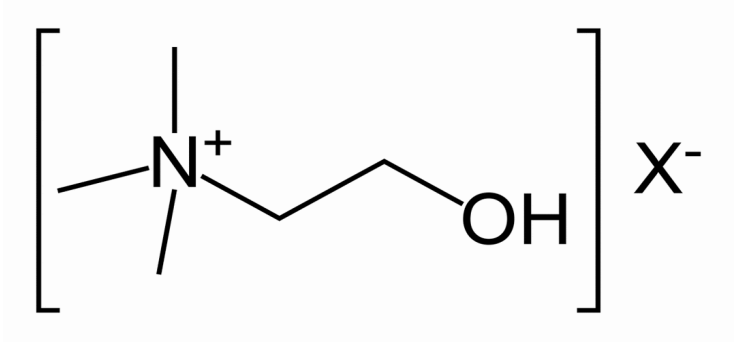
Trimethylamine N-oxide (TMAO)

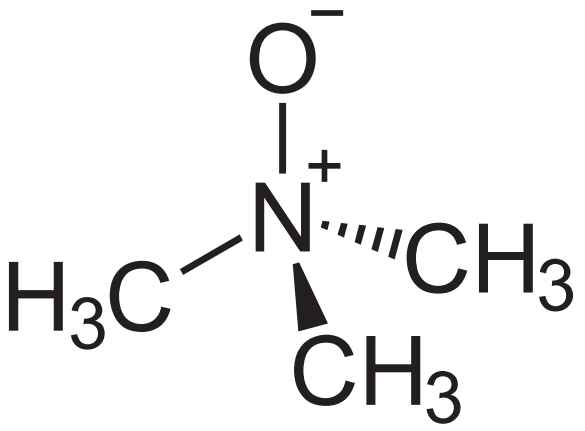
Important compound in deep-sea fish

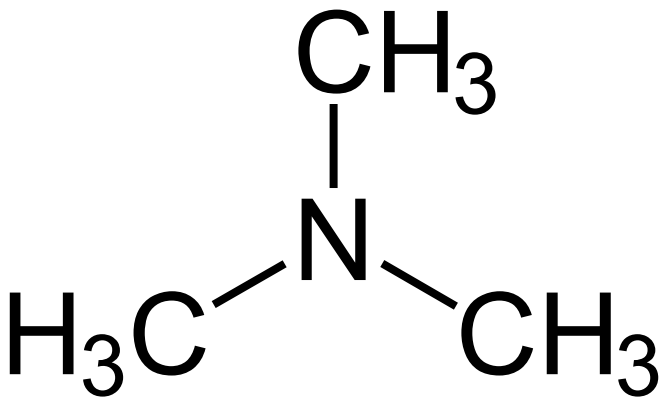
- Stabilizes protein molecules against effects of pressure and osmolarity

Found in other animals, and its metabolism is known

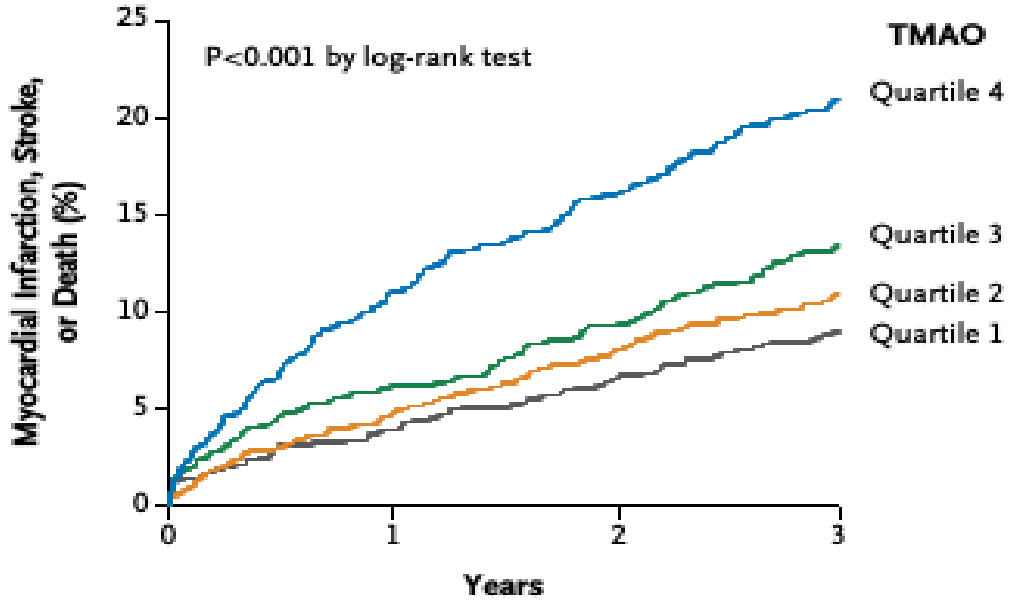
- It's a metabolic product of choline, by way of trimethylamine







ASCVD events and TMAO

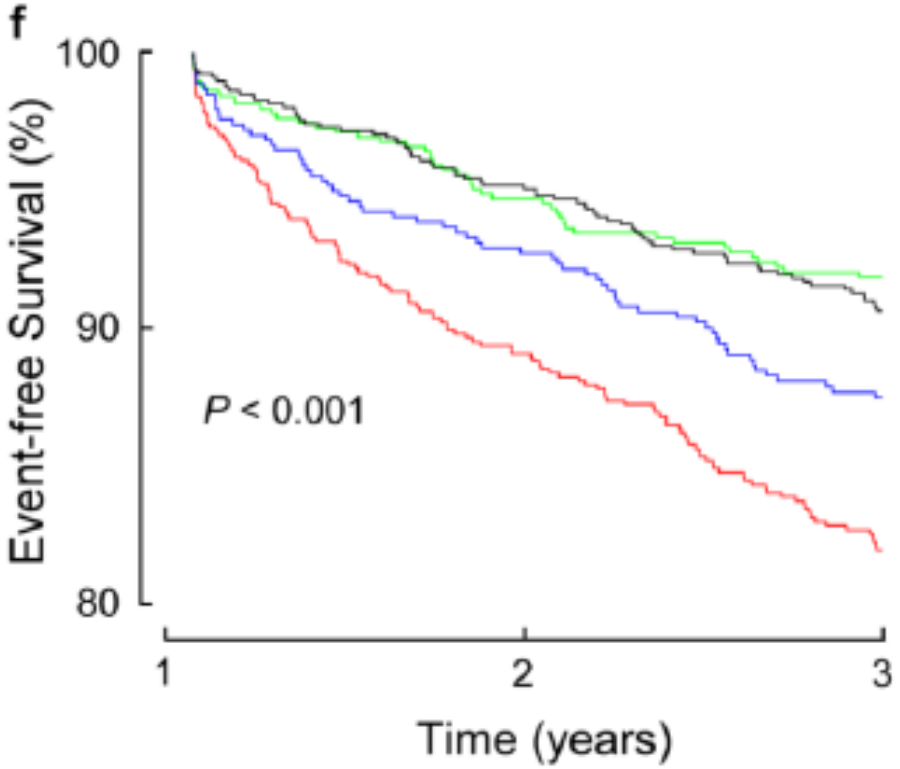


No. at Risk				
Quartile 1	1001	933	869	827
Quartile 2	998	940	884	843
Quartile 3	1003	938	888	835
Quartile 4	1005	913	849	791

Figure 2. Kaplan–Meier Estimates of Major Adverse Cardiovascular Events, According to the Quartile of TMAO Level.

Data are shown for 4007 participants in the clinical-outcomes study. The P value is for all comparisons.

ASCVD events and TMAO



Carnitine	TMAO	Unadjusted HR (95%)	Adjusted HR (95%)
High	Low	0.9 (0.6–1.4)	0.8 (0.5–1.3)
Low	Low	1.0 (Reference)	1.0 (Reference)
Low	High	1.6 (1.2–2.0)	1.3 (1.02–1.7)
High	High	2.5 (1.8–3.4)	2.1 (1.5–2.8)

Of mice and TMAO

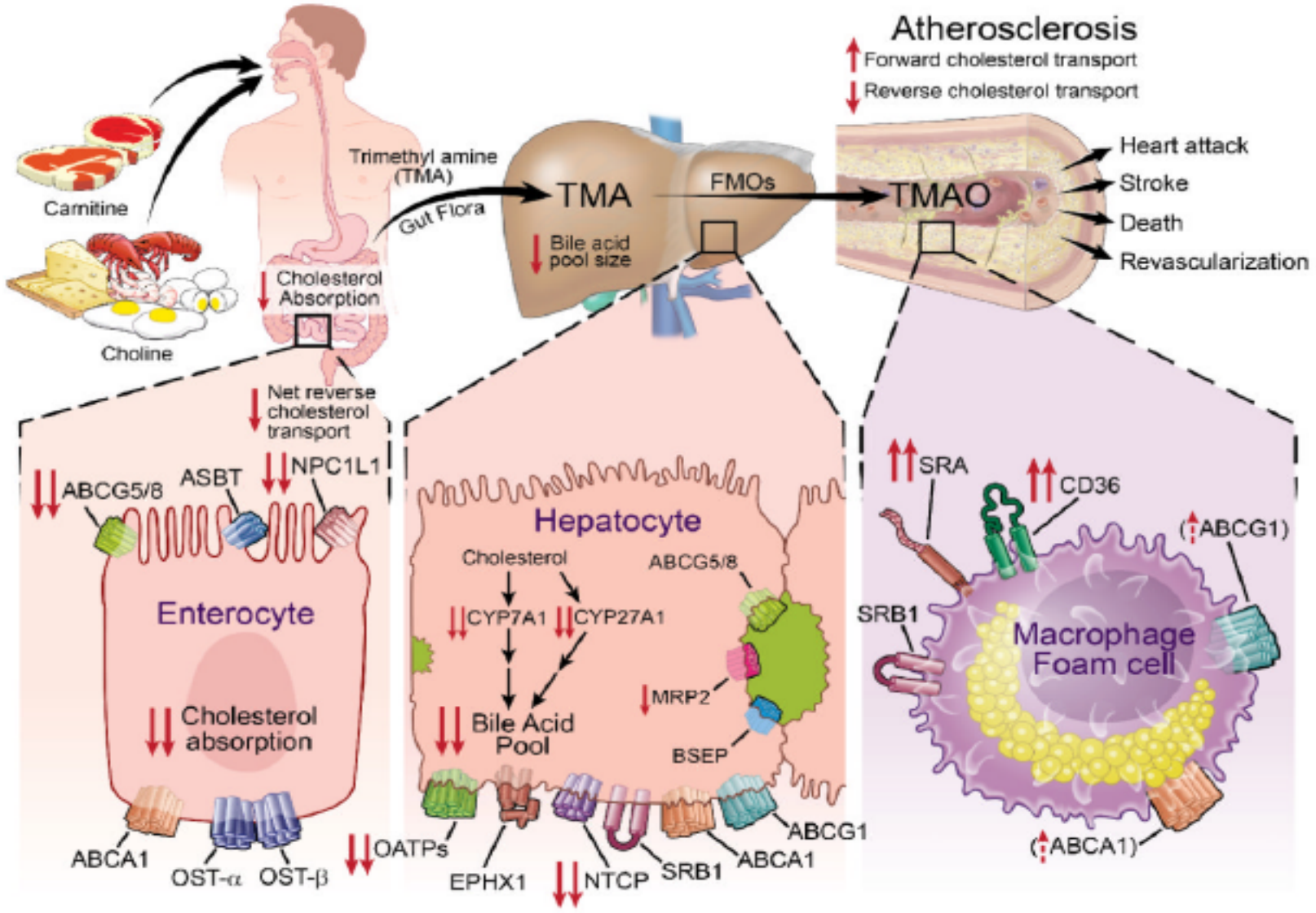
ASCVD-prone mice (APM) given TMAO show accelerated atherosclerosis (AAS)

APM fed choline or carnitine have TMAO in blood -> AAS

Germ-free APM, or those given antibiotics, + choline or carnitine → No AAS!

Choline or carnitine in diet is converted to TMAO by way of gut bacteria

TMAO & ASCVD

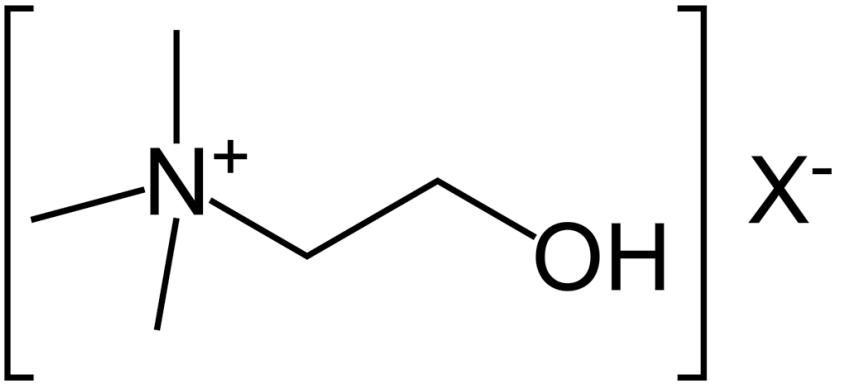

Atherosclerosis
Forward cholesterol transport
Reverse cholesterol transport
Heart attack
Stroke
Death
Revascularization
Carnitine
Choline
Trimethyl amine (TMA)
Gut Flora
TMA
FMOs
TMAO
Bile acid pool size
Cholesterol Absorption
Net reverse cholesterol transport
ABCG5/8
ASBT
NPC1L1
Enterocyte
Cholesterol absorption
ABCA1
OST-α
OST-β
Hepatocyte
Cholesterol
CYP7A1
CYP27A1
Bile Acid Pool
ABCG5/8
MRP2
BSEP
OATPs
EPHX1
NTCP
SRB1
ABCA1
ABCG1
SRA
CD36
ABCG1
SRB1
Macrophage Foam cell
ABCA1

Choline and phosphatidylcholine

Important compounds in the assembly of cell membranes and many other metabolic pathways

Essential to human development and health

Abundant in egg yolks, adequate amounts in many other foods (meat, fish, poultry, dairy)



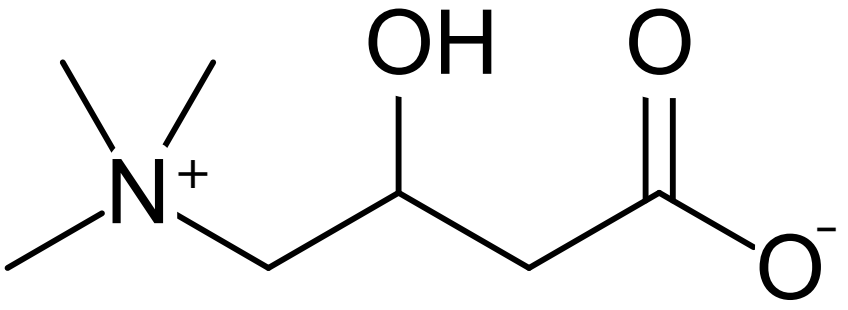
Carnitine

Necessary for movement of fatty acids into mitochondria in human cells

Synthesized in humans from other substrates

- Not required in diet

Abundant in animal-based foods, especially red meats



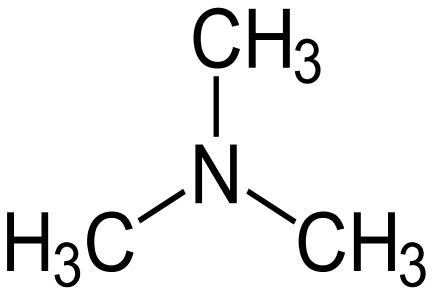
Product	Quantity	Carnitine
Beef steak	100 g	95 mg
Ground beef	100 g	94 mg
Pork	100 g	27.7 mg
Bacon	100 g	23.3 mg
Tempeh	100 g	19.5 mg
Cod fish	100 g	5.6 mg
Chicken breast	100 g	3.9 mg
American cheese	100 g	3.7 mg
Ice cream	100 ml	3.7 mg
Whole milk	100 ml	3.3 mg
Avocado	one medium	2 mg[20]
Cottage cheese	100 g	1.1 mg
Whole-wheat bread	100 g	0.36 mg
Asparagus	100 g	0.195 mg
White bread	100 g	0.147 mg
Macaroni	100 g	0.126 mg
Peanut butter	100 g	0.083 mg
Rice (cooked)	100 g	0.0449 mg
Eggs	100 g	0.0121 mg
Orange juice	100 ml	0.0019 mg

Trimethylamine (TMA)

TMA is a simple volatile molecule that gives rotting fish their smell

Normally only present in small quantities in humans

However, if we happen to eat foods with choline, PC, carnitine, or betaine, certain gut bacteria can turn them into TMA

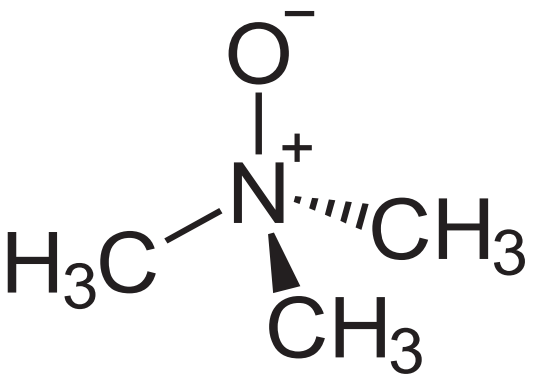


Trimethylamine N-oxide (TMAO)

Promotes ASCVD by several mechanisms

- Enhances forward cholesterol transport, inhibits reverse cholesterol transport
- Activates macrophages into foam cells

Oops!



My take on meat and health

We now have biologically plausible explanations for the deleterious health effects of red meat

One, involving choline-associated compounds, is mediated through TMAO

- Choline and carnitine supplements must also be viewed with caution

The other, involving proteins and especially branched-chain amino acids, works via nutrient-signaling pathways that control cell proliferation, apoptosis, etc, with profound implications for health and longevity

Conclusions – Meat and Mortality

Increased mortality is associated with increasing red meat consumption

- In the US, both unprocessed and processed red meats are implicated

Death due to cancer and heart disease are specifically implicated, as well as all-cause mortality

White meat, by contrast, is inversely associated with mortality, i.e., may have a protective effect

Recommendations

Limit red meat consumption to no more than two servings per week

- Processed meats are especially worrisome

If you desire to eat meat, rely on poultry as your main source

- We'll talk about fish separately

Carnitine and choline supplements should be avoided