THE WOMEN'S HEALTH INITIATIVE

Plenary 3: Multi-omics resources and applications to WHI

Chair: Nora Franceschini, University of North Carolina

Plenary 3: Multi-omics resources and applications to WHI

New Multi-omics SIG

Co-Chairs

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Goals

- Generate new collaborations for –omics-related studies on women's health and aging-related traits
- Expand/generate new interest among WHI investigators in the use of –omics data
- Provide information about WHI resources & develop standardized quality control and methods for data use
- Forum for discussion of ideas and methods applied to this data

Multi-omics biomarkers

- DNA methylation
- Proteomics
- Metabolomics
- Gene expression
- CHIP
- Microbiome

Examples of applications for research

- Nutrition
- Environmental exposures
- TOPMed & AS

Diet, Epigenetics, and Clinical Outcomes

WHI Annual Investigator Meeting
May 1, 2025

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WOMEN'S HEALTH INITIATIVE

Diet is a major modifiable risk factor in human health and disease risk

- Better diet quality associated with lower risk of mortality and chronic diseases
- Varying responses to diet depending on
  - Life stage
  - Health or disease status
  - Genetics/omics

Associations of dietary cholesterol and fat, blood lipids, and risk for dementia in older women vary by APOE genotype

Michelle M. Dunk[1,2] | Jie Li[3,4,5] | Simin Liu[3,4] | Ramon Casanova[6] | Jiu-Chiuan Chen[7] | Mark A. Espeland[6,8,9] | Kathleen M. Hayden[9] | JoAnn E. Manson[10,11] | Stephen R. Rapp[9,12] | Aladdin H. Shadyab[13] | Linda G. Snetselaar[14] | Linda Van Horn[15] | Robert Wild[16] | Ira Driscoll[1,17]

- Precision nutrition aims to develop targeted nutrition recommendations customized to prevent and/or manage chronic diseases in groups of susceptible individuals
  - RCTs are needed to support precision nutrition recommendations
  - Costly due to need for large sample sizes and long-term follow up

Biomarkers of Aging:
Potential Outcomes for Precision Nutrition Interventions

- Chronological age is the largest risk factor many chronic diseases and disabilities
- Aging is heterogeneous
- Biological aging - morphological and functional decline affecting the aging organism
  - Lack of a consensus on how to measure biological aging
  - Potential biomarkers of aging are emerging
    - Quantify hallmarks of aging: epigenetic alterations
    - Long-term studies are needed linking aging biomarkers with progression in clinical phenotypes

Goals: - Increase healthspan through precision nutrition approaches targeting biological aging

- Validation of epigenetic biomarkers

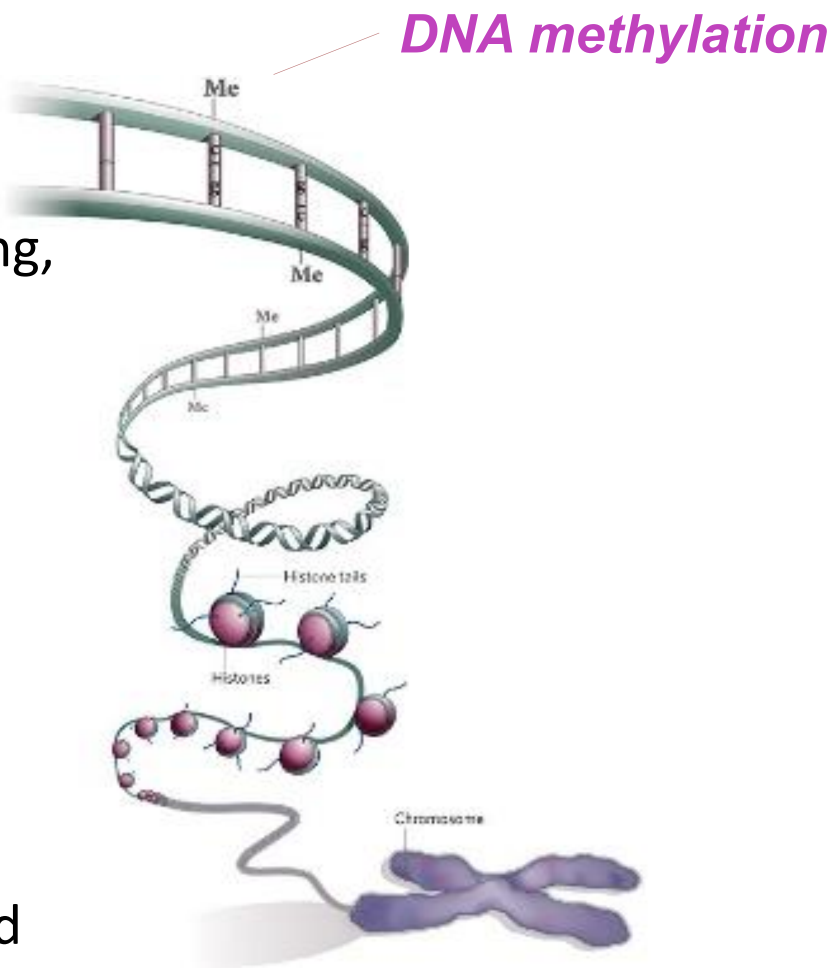
DNA methylation

- Reversible epigenetic modification (methyl transfer)
- Important for chromatin structure, transcription factor binding, and regulation of gene expression
- Epigenetic alterations are hallmark of aging
- Interface between genetics and environment

  Influenced by many factors

  Aging Lifestyle Environmental Genetics

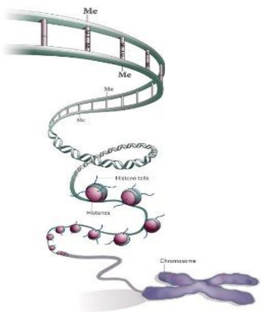
  Dietary factors – specific nutrients, fiber intake, alcohol intake
- Predictive of morbidity and mortality risk
- Potential mechanism underlying link between diet, aging, and disease



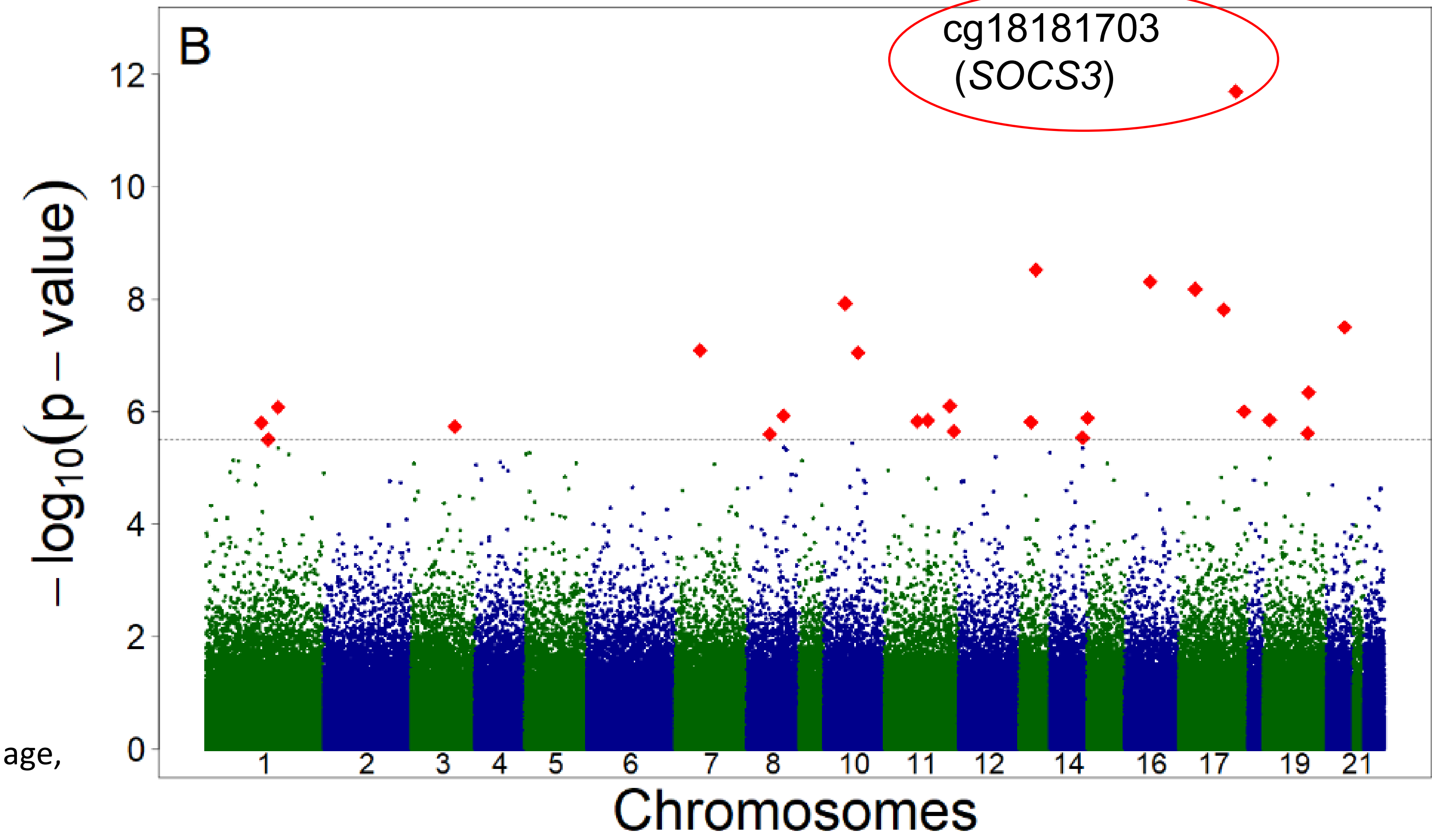
THE WOMEN'S HEALTH INITIATIVE

Diet Quality EWAS identifies biologically relevant gene

Diet quality:
AHEI + MDS



Linear regression adjusting for age, sex, and energy intake



Meta-analysis: 5 cohorts: ARIC, FHS, GOLDN, MESA, RS, including 6,662 European ancestry participants

Cohorts for Heart and Aging Research in Genomic Epidemiology

The Women's Health Initiative

Ma et al. Circ Genom Precis Med 2020

Diet quality EWAS top hit biologically relevant cg18181703 (SOCS3)

- Suppressor of cytokine signaling 3 (SOCS3)
  - Major regulator of inflammation
  - Involved in control of energy metabolism
    - Leptin and insulin signaling
  - Inhibition of SOCS3 promising therapeutic approach to improve cardiometabolic health

Pedroso et al. *Hormones* 2018

- cg18181703 methylation ~
  -

					Meta-Analysis in All EA Participants			
CpG	CHR	Position	Gene	Diet	β	SE	*P* Value	Direction
cg18181703	17	76354621	*SOCS3*	AHEI	0.004	0.001	2.0×10^{-12}	+, +, +, +, +
				MDS	0.004	0.001	3.5×10^{-10}	+, +, +, +, +

  - Better cardiometabolic health (↓BMI, ↓Risk of diabetes)
  - Lower risk for all-cause mortality (P=5.7×10^{-15})

Ma *et al. Circ Genom Precis Med* 2020

- Epigenetic modifications potentially mediate associations between diet and cardiometabolic health

THE WOMEN'S HEALTH INITIATIVE

Epigenetic Aging

- An individual's degree of aging based on patterns of DNA methylation
- Growing number of epigenetic aging measures

Biomarker:	Hannum et al. (2013)	Horvath (2013)	PhenoAge Levine et al. (2018)	GrimAge Lu et al. (2019)	DunedinPACE Belsky et al. (2022)
# of sites:	71	353	513	1,030	20,000
Tuned to predict:	Chronological age		Survival – biological age based on estimated risk of death		Pace of Aging – per-year decline in organ-system integrity **Based on longitudinal decline in organ-system integrity across two decades**

- Advanced epigenetic aging considered to reflect faster rate of biological aging
- Associated with:

Dietary factors
Other lifestyle
Cardiometabolic measures
Genetics

AgeAccelGrim

		n	bicor	p
Diet	log2(Total energy)	3700	-0.02	0.15
	Carbohydrate	3700	-0.12	4E-13
	Protein	3700	-0.01	0.39
	Fat	3700	0.09	2E-8
	log2(1+Red meat)	3700	0.06	3E-4
	log2(1+Poultry)	3700	0.03	0.08
	log2(1+Fish)	3700	0.00	0.87
	log2(1+Dairy)	3700	-0.09	1E-7
	log2(1+Whole grains)	3700	-0.07	2E-5
	log2(1+Nuts)	3700	-0.02	0.15
	log2(Fruits)	3700	-0.10	1E-10
	log2(Vegetables)	3700	-0.08	7E-7
Dietary biomarkers	Retinol	2267	-0.01	0.49
	Mean carotenoids	2266	-0.16	9E-39
	Lycopene	2267	-0.07	6E-4
	log2(alpha-Carotene)	2267	-0.28	4E-44
	log2(beta-Carotene)	2266	-0.22	5E-28
	log2(Lutein+Zeaxanthin)	2267	-0.14	9E-12
	log2(beta-Cryptoxanthin)	2267	-0.22	2E-26
	log2(alpha-Tocopherol)	2267	-0.06	3E-3
	log2(gamma-Tocopherol)	2267	0.14	2E-11
Measurements	log2(C-reactive protein)	2809	0.28	2E-52
	log2(Insulin)	4042	0.16	2E-26
	log2(Glucose)	4144	0.12	2E-14
	log2(Triglyceride)	4148	0.11	5E-13
	Total cholesterol	4148	0.01	0.65
	LDL cholesterol	4084	0.00	0.83
	HDL cholesterol	4145	-0.10	1E-10
	log2(Creatinine)	2748	0.03	0.07
	Systolic blood pressure	4177	0.07	9E-7
	Diastolic blood pressure	4178	-0.01	0.36
	BMI	4145	0.14	1E-20
	log2(Waist / hip ratio)	4037	0.19	4E-34
Life style	Education	4143	-0.09	2E-9
	Income	4054	-0.07	2E-6
	log2(1+Exercise)	3914	-0.10	3E-10
	Current smoker	2321	0.44	5E-113
	log2(1+Alcohol)	3700	-0.04	0.02

Adapted from Lu et al., *Aging* 2019

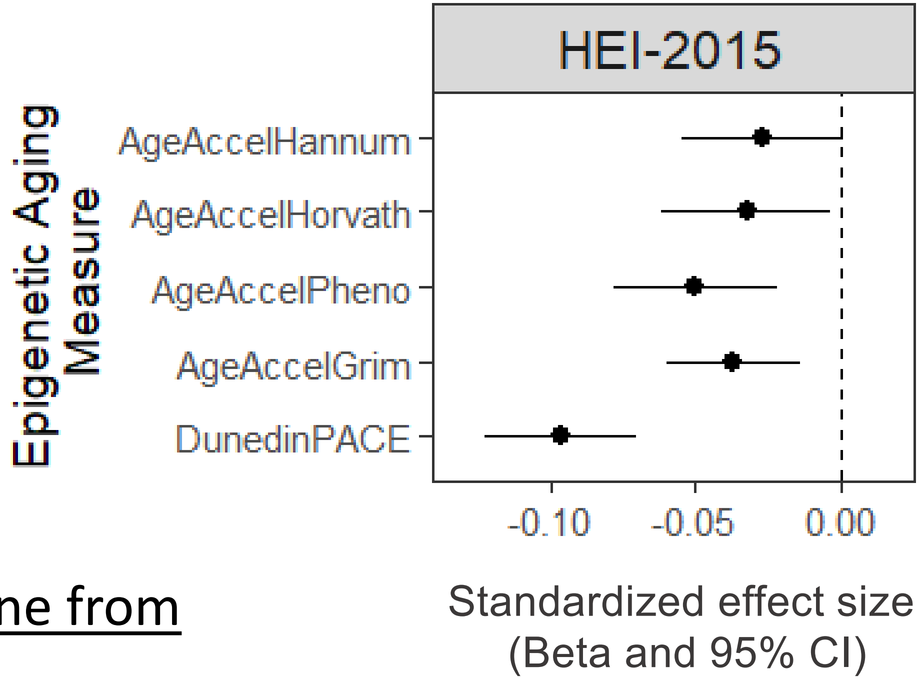
Epigenetic Aging and Diet Quality

5,389 participant blood samples from the Women's Health Initiative with DNA Methylation data
- n=882 from AS311
- n=2,107 from BAA23
- n=2,400 from EMPC

→ Missing dietary data or implausible dietary intake (n=208)
Missing covariate data (n=541)
Participant in more than one study (n=140)

↓

4,500 WHI participants
(primary analysis)



<u>Existing DNA methylation data from blood collected at baseline from</u>

- AS311 (Bladder Cancer and Leukocyte Methylation Study)
- BAA23 (The Integrative Genomics for Risk of Coronary Heart Disease and Related Phenotypes)
- EMPC (Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease Study)

<u>Model adjusting for:</u> age, race and ethnicity, education, smoking status and pack-years of smoking, physical activity, WHI ancillary (random and fixed effect), and leukocyte proportions

Reynolds et al. *J Acad Nutr Diet* 2024

Next step:
Validation of epigenetic aging biomarker with clinical phenotype

- **Assess epigenetic aging as a marker of biological processes mediating the relationship between diet and transition to frailty.**
- *Hypothesis*: The benefits of good diet quality on preventing frailty are partially explained by slower epigenetic aging.

Frailty

Frailty

- State of vulnerability to adverse outcomes
- Major risk factor for falls, disability, hospitalization, loss of independence, death
- Fried's frailty phenotype
  - Syndrome based on a cluster of signs and symptoms that commonly occur in vulnerable older adults

Fried Frailty Phenotype	Frail ≥ *3 of 5 components:* Unintentional weight loss Weakness Exhaustion/fatigue Slowness in walking Physical inactivity

A better understanding of how to prevent or delay frailty is critically important to lessen individual and healthcare burdens in the growing population of older adults

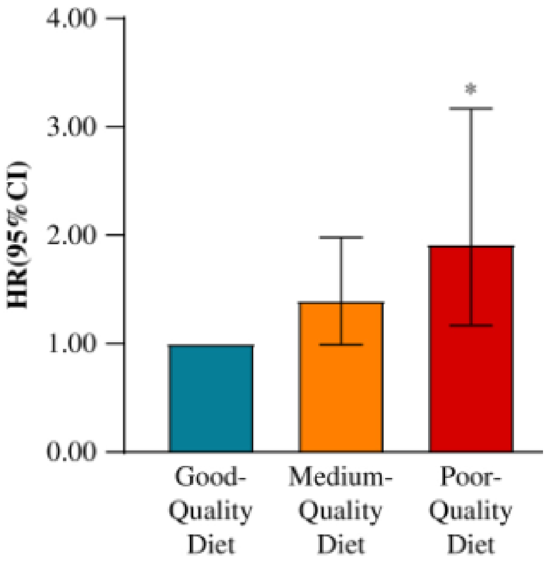
THE WOMEN'S HEALTH INITIATIVE

Diet quality and protein intake may be an intervention target for frailty

<u>Higher risk of frailty associated with:</u>

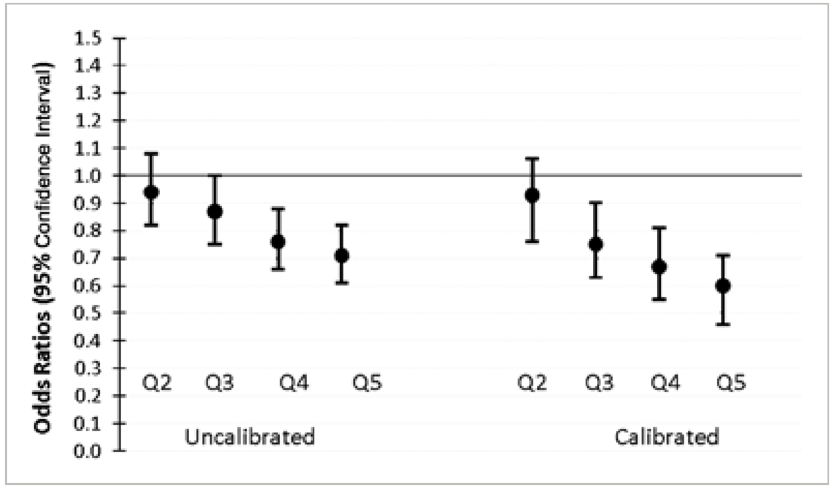
- Poorer diet quality   Lower vegetable protein intake
    - 2,154 older adults in the Health ABC Study with 4-year follow-up
    - No association with energy intake or total protein intake

Hengeveld et al. *J Am Geriatr Soc* 2019

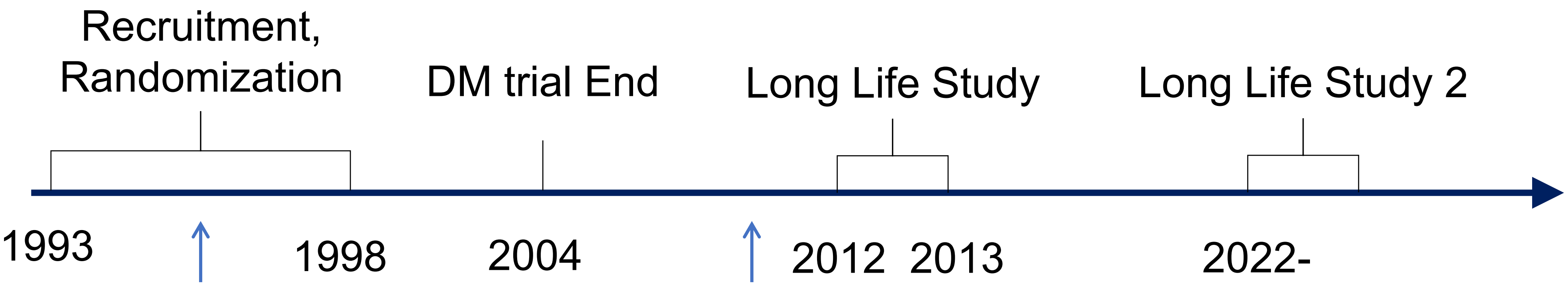


- Lower protein intake
    - 24,417 WHI OS participants - Baseline to Annual Visit 3
    - Biomarker-calibrated estimates of energy and protein intake derived to address dietary self-report error
        - Corrected for measurement error using regression calibration equations estimated from objective measures of total energy expenditure (doubly labeled water) and dietary protein (24-hour urinary nitrogen)
    - The strength of the association was underestimated using uncalibrated measures

Beasley et al. *J Am Geriatr Soc* 2010



WHI and Modified Fried Frailty



Baseline Data availability

- Diet (FFQ + Supplement use)
- Physical Frailty Phenotype
- DNA methylation data

AS311 (Bhatti) BAA23 (Assimes)

EMPC (Whitsel)

- N=1,652 with ~12-year follow up data (Rand-36)
- Free of frailty at baseline

64% robust, 36% prefrail

- Mean (SD) DunedinPACE = 1.01 (0.12)

Women's Health Initiative Observational Study (WHI-OS) frailty measure

Scoring: ≥3/5 criteria met indicates frailty; 1-2/5 indicates pre-or-intermediate frailty; 0/5 indicates non-frail.

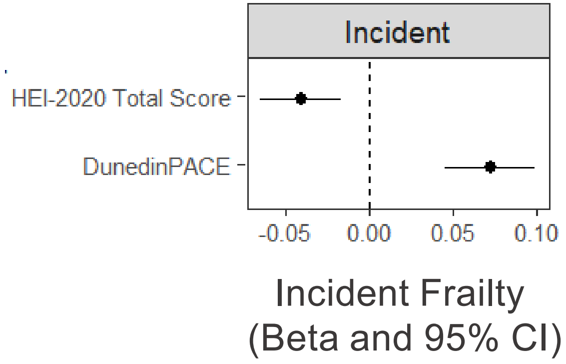
Frailty Criterion	Definition
Slowness / weakness	**Meets criteria for slowness / weakness if:** Score of <75 out of 100 on the Rand-36 Physical Function Scale[1]: Includes 10 items measuring whether health limits physical function. Note: this is scored as 2 criteria.
Poor endurance / exhaustion	**Meets criteria for poor endurance / exhaustion if:** Score of <55 out of 100 on the Rand-36 Vitality Scale2, using the following questions: Over past 4 weeks: • *Did you feel worn out?* • *Did you feel tired?* • *Did you have a lot of energy* • *Did you feel full of pep?*
Physical activity	**Detailed physical activity questionnaire:** Assess frequency and duration of walking and mild, moderate, and strenuous activities. Kcal of weekly energy expenditure calculated (metabolic equivalent task hours score = kcal/wk x kg), and those in lowest quartile score as meeting criteria for this component.
Unintentional weight loss	**Meets criteria for weight loss if:** Lost >5% body weight in last 2 years, and reported "Yes" to the question, "In the past two years, did you lose five or more pounds not on purpose at any time?" Equipment: scale for body weight; stadiometer for height.

[1] https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form.html
2 See energy / fatigue in Table 2: https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/scoring.html

References:

Woods NF, LaCroix AZ, Gray SL, et al. Frailty: emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study [published correction appears in J Am Geriatr Soc. 2017 Jul;65(7):1631-1632]. J Am Geriatr Soc. 2005;53(8):1321-1330.

Epigenetic aging mediates association between Diet Quality and Incident Frailty



Epigenetic pace of aging (DunedinPACE)
(Baseline)

17% Mediated

Indirect effect of diet quality on frailty through DunedinPACE
-0.007

Diet quality (HEI-2020 from FFQ)
(Baseline)

Direct effect
-0.034

Incident Frailty
Baseline to ~12 years follow-up

Linear mixed model <u>covariates:</u>
- Age
- Race
- Education
- Smoking status
- Pack-years
- Physical activity
- WHI ancillary study (fixed and random effect)
- DNAm-based estimates of leukocyte proportions

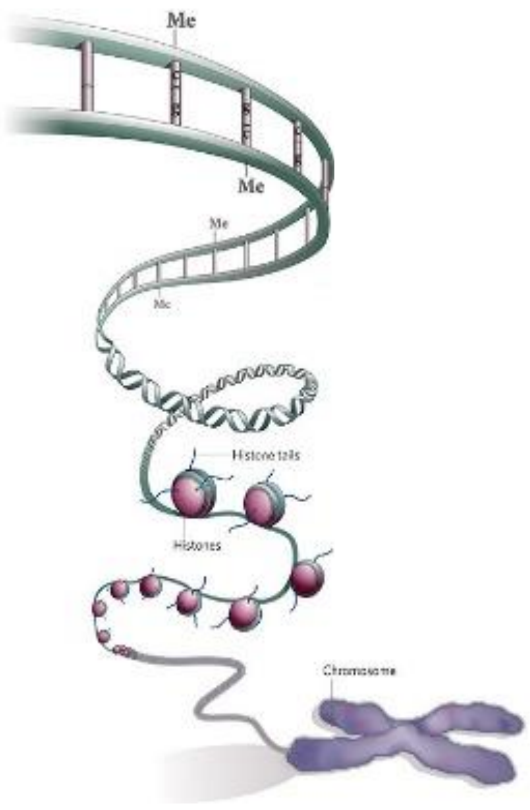
Casual mediation analyses

Reynolds et al. unpublished

Summary

DNA methylation is potential mediator of effects of diet on health and aging

- Tool to understand dietary effects on health and aging biology
  - Diet quality-related methylation of *SOCS3*
    - Impacts cardiometabolic health and mortality risk
  - Higher diet quality was associated with lower risk of frailty
    - Epigenetic aging biomarker partially mediates association
      - DunedinPACE



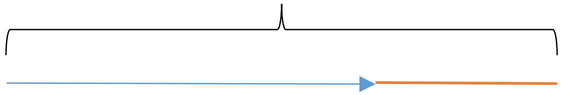
Limitations

- Other influences to epigenetics and frailty
- Diet quality and epigenetic data only at baseline
- Self-reported FFQ data
- Modified Fried Frailty Phenotype

Future Directions

- Utilize biomarker-corrected dietary intake measures
- Incorporate dietary supplement intake as dietary exposure
  - Total Nutrient Index (TNI)
- Examine diet-related changes in epigenetic aging trajectories
- Account for genetic influences to aging and metabolism
- Quantify association between diet quality, epigenetic aging, and other age-related clinical outcomes
- Dietary effects on other aging biomarkers
- Design and test precision nutrition interventions to increase healthspan

Lifespan



Healthspan -
Time spent free from
chronic disease and
disability

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Wake Forest Claude Pepper
Older Americans Independence Center

NIH National Institute on Aging

Cohorts for Heart and Aging Research in Genomic Epidemiology

Women's Health Initiative

Health Initiative

Environmental Epigenetics & Chronic Disease Risk: Enhancing Risk Prediction to Address Disparities

WHI INVESTIGATOR MEETING

ANDRES CARDENAS, PHD

MAY 1, 2025

Stanford MEDICINE | Department of Epidemiology & Population Health

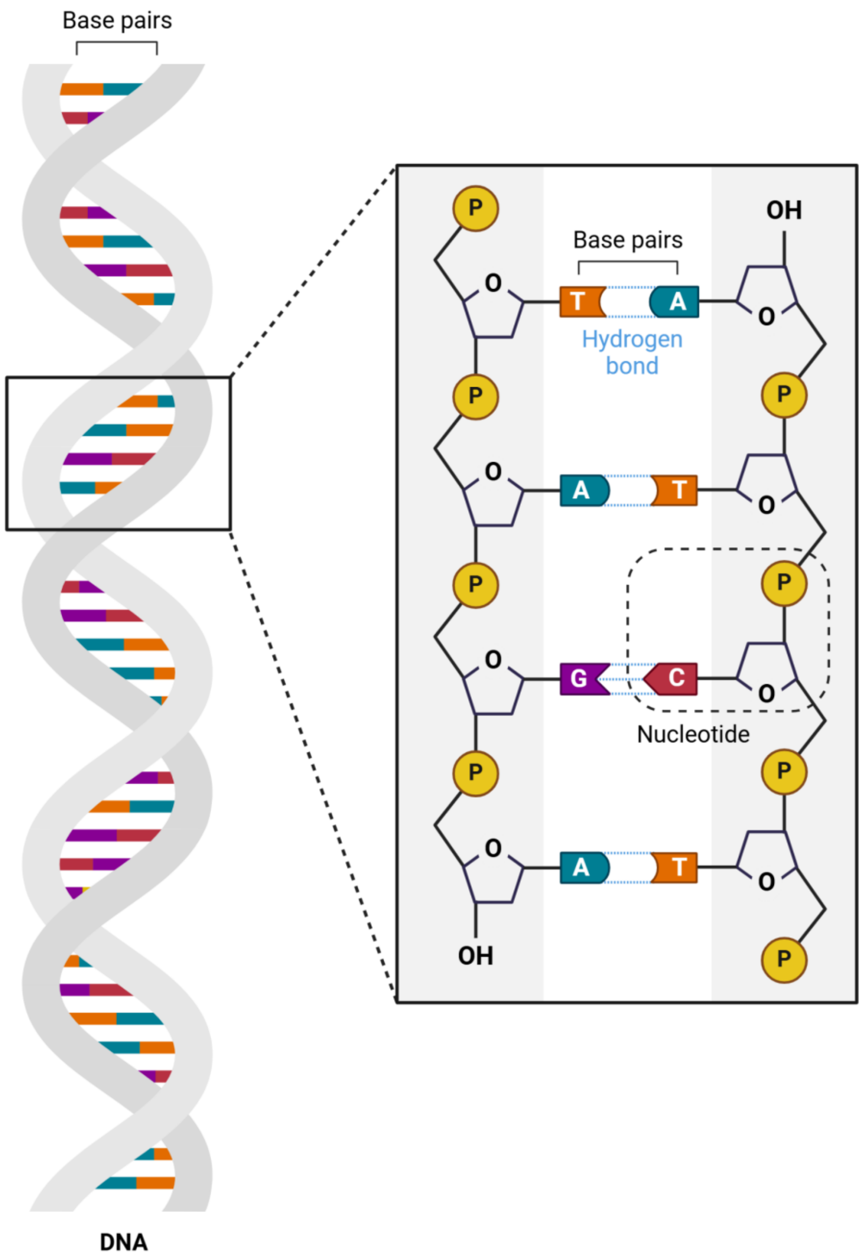
DNA

Central Molecule of Life

- Instructions of life
- The code is the same across every cell in your body
- The code is 99.9% identical across individuals

MZ twins

Okada, HC., *et al*. Plastic & Reconstructive Surgery 132.5(2013):1085



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Epigenetics

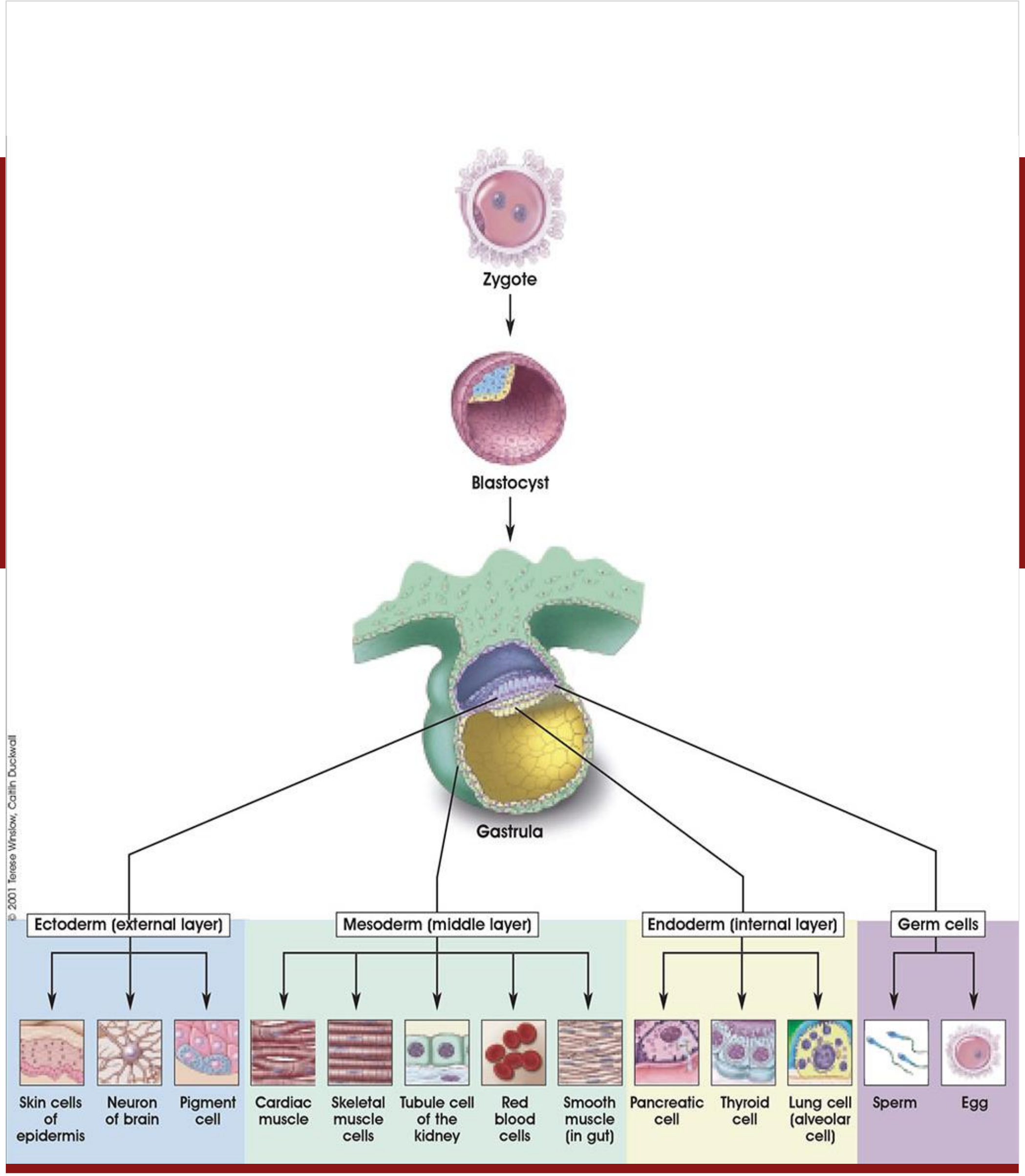
Changes in gene expression that:

- Do not depend on the DNA sequence
- Can be stable
- May persist (mitotically stable)

01 **Tissue specific**
Same genome ≠ epigenomes

02 **Epigenetics contribute to tissue differentiation**

03 **Each cell-type has a unique epigenetic signature**



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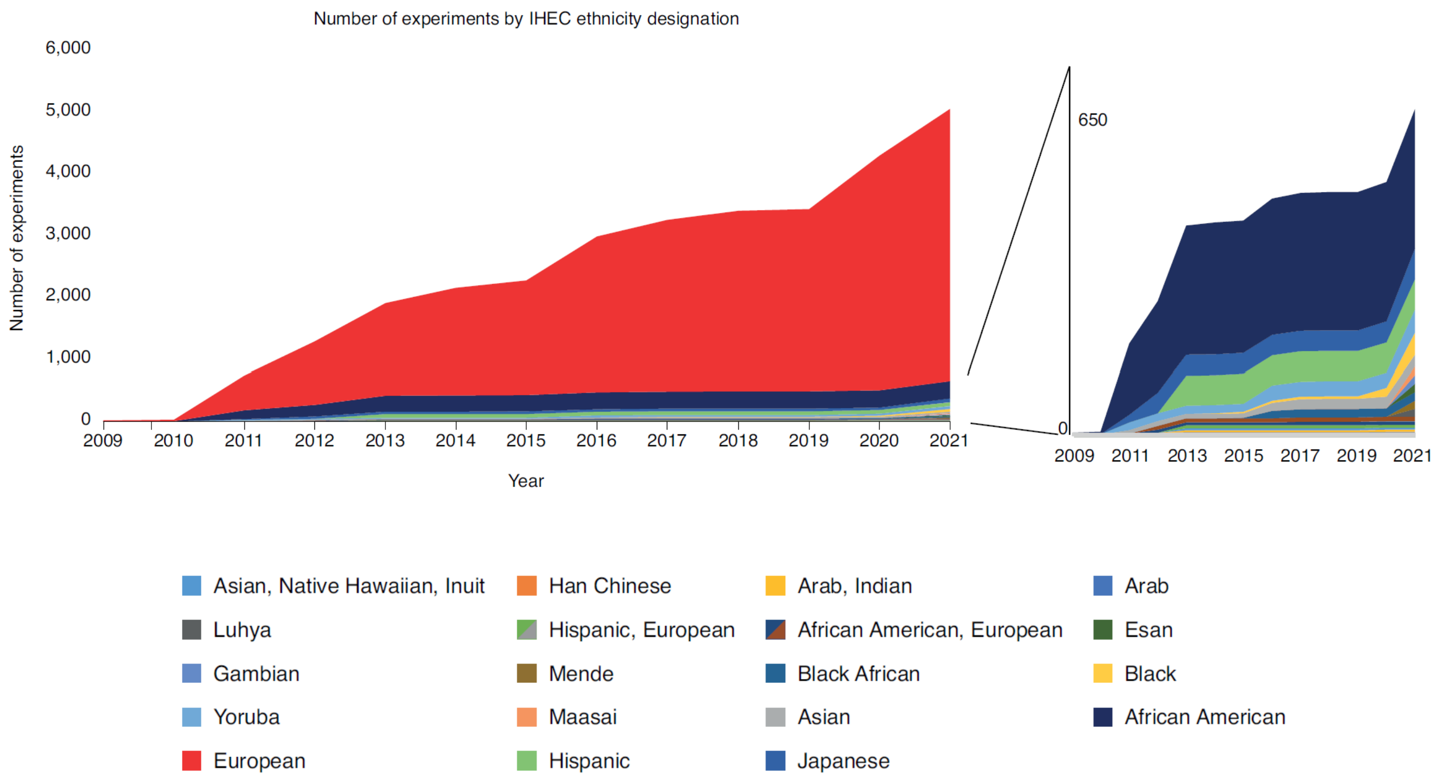
Diversity of epigenetic studies



Breeze, CE., et al. "The missing diversity in human epigenomic studies." *Nature Genetics*. 54.6 (2022): 737-739

Epigenetic Biomarkers of Cardiovascular Disease in African American Women

R01HL175681 (MPI: Franceschini/Cardenas)

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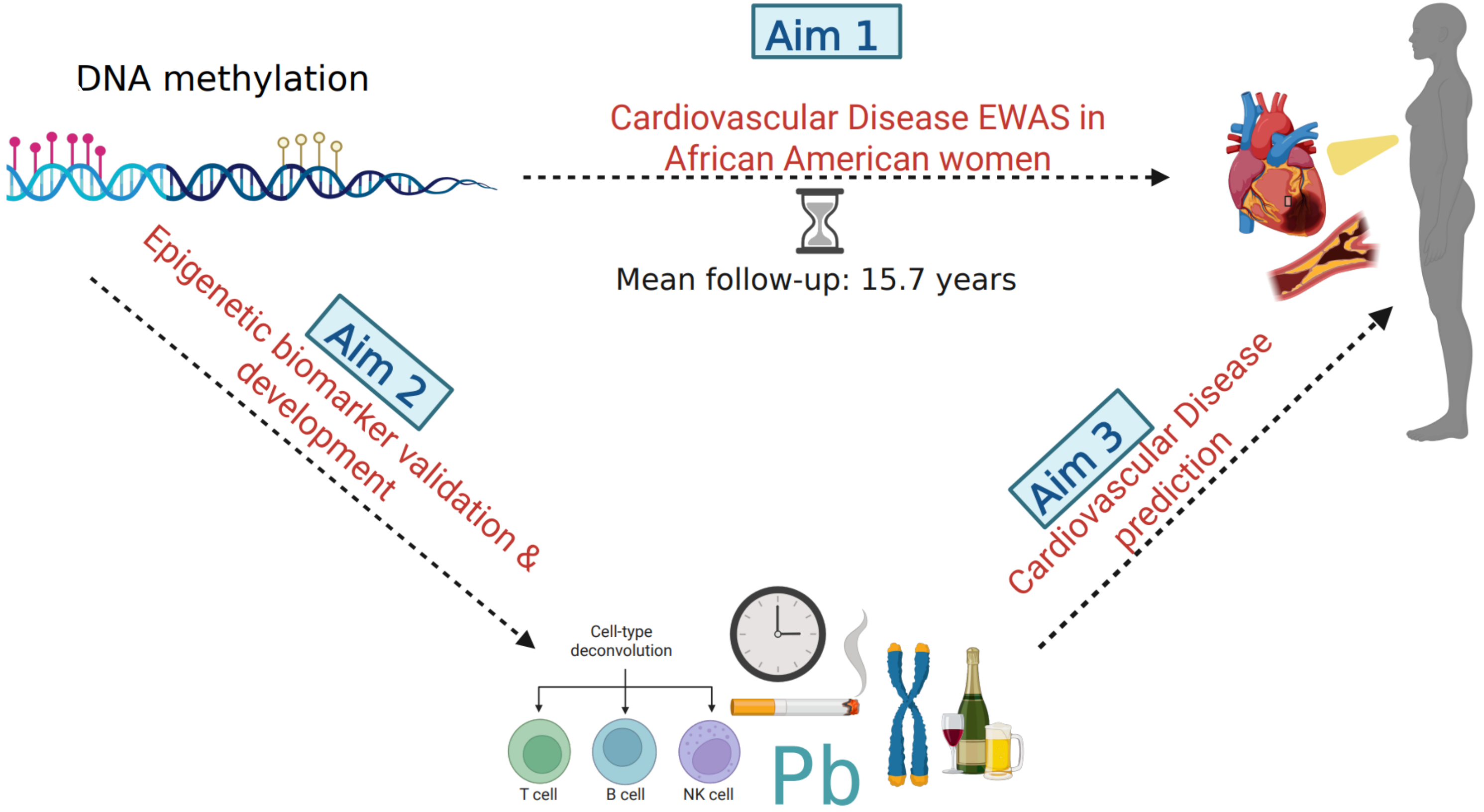
Overview of the Proposed Study Aims

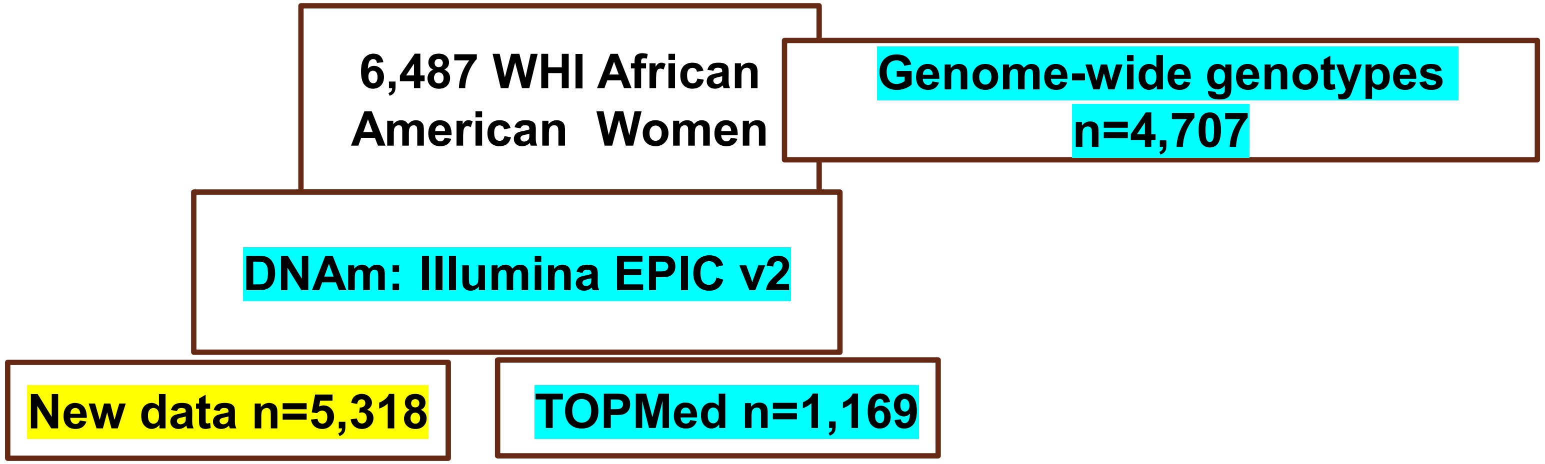


Aim 1: Epigenetic discovery CVD EWAS

Aim 2: Epigenetic biomarker validation

Aim 3: Epigenetic biomarker prediction

Samples for Primary and Secondary Outcomes

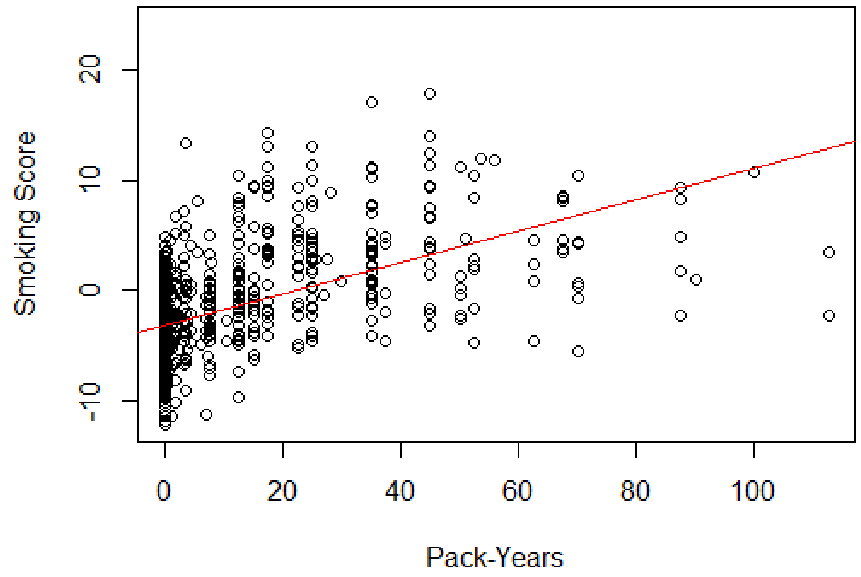


	N*	Primary outcome: Incident fatal/non-fatal CHD	Total Stroke	Composite CHD and total stroke
Aims 1, 2 and 3	6,484	877	557	1,303
Aim 3.b	4,707	788	495	1,162

*Includes n=1,169 DNAm samples already available through TOPMed (EPIC v1). **412 of total strokes are ischemic strokes. For composite outcomes, we only included the first event so the total number of events is not a sum.

WHI Data – Smoking Biomarker

- Smoking DNA methylation Biomarker (EpiSmoker); *r*=0.54



- Associations of EpiSmoker and self-reported pack-years with incident CHD

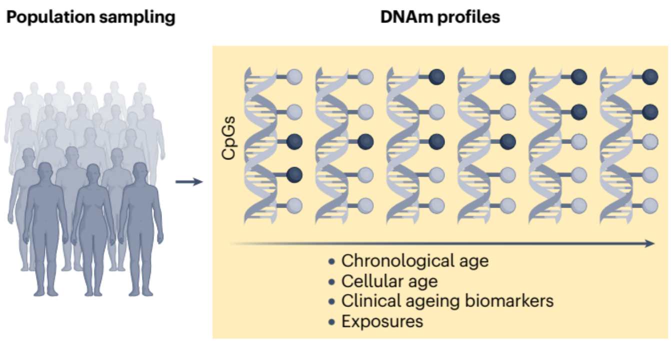
Predictor of incident CHD	HR (95% CI)
DNAm smoking score quantitative	1.09 (1.05, 1.13)
DNAm smoking score tertiles	
3rd tertile (ref=1st)	1.98 (1.27, 3.10)
2nd tertile (ref=1st)	1.19 (0.73, 1.92)
Self-reported pack-years	1.01 (1.00, 1.02)

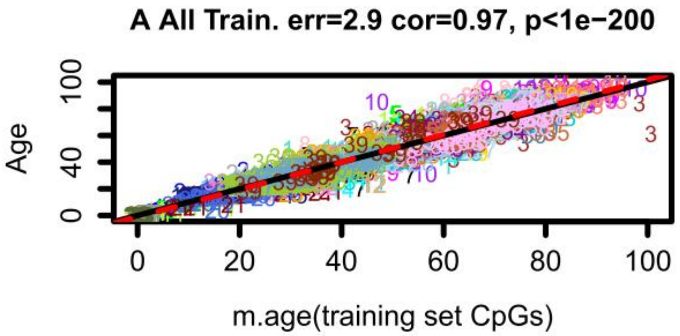
n=969 WHI women/multiethnic sample

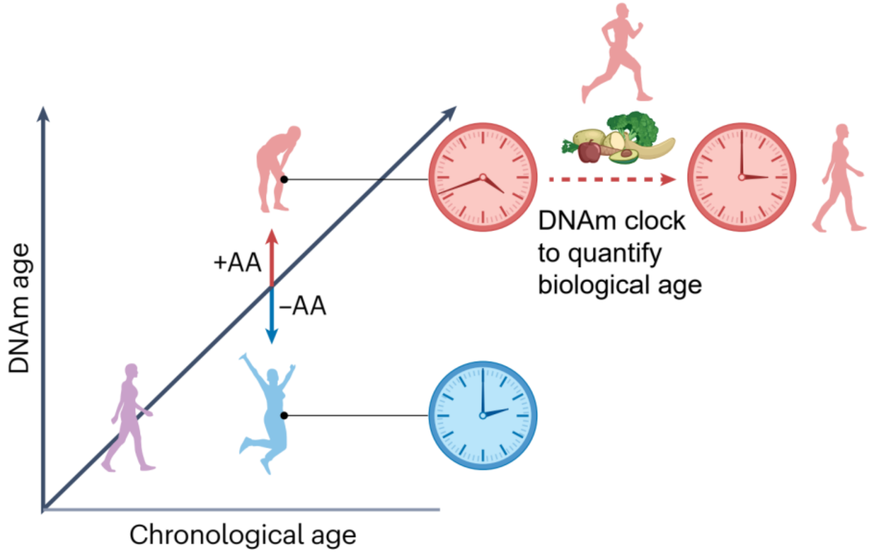
Epigenetic Age (DNAm Age)

A biological epigenetic clock

Epigenetic clock correlated with chronological age
Using DNA methylation of many genes



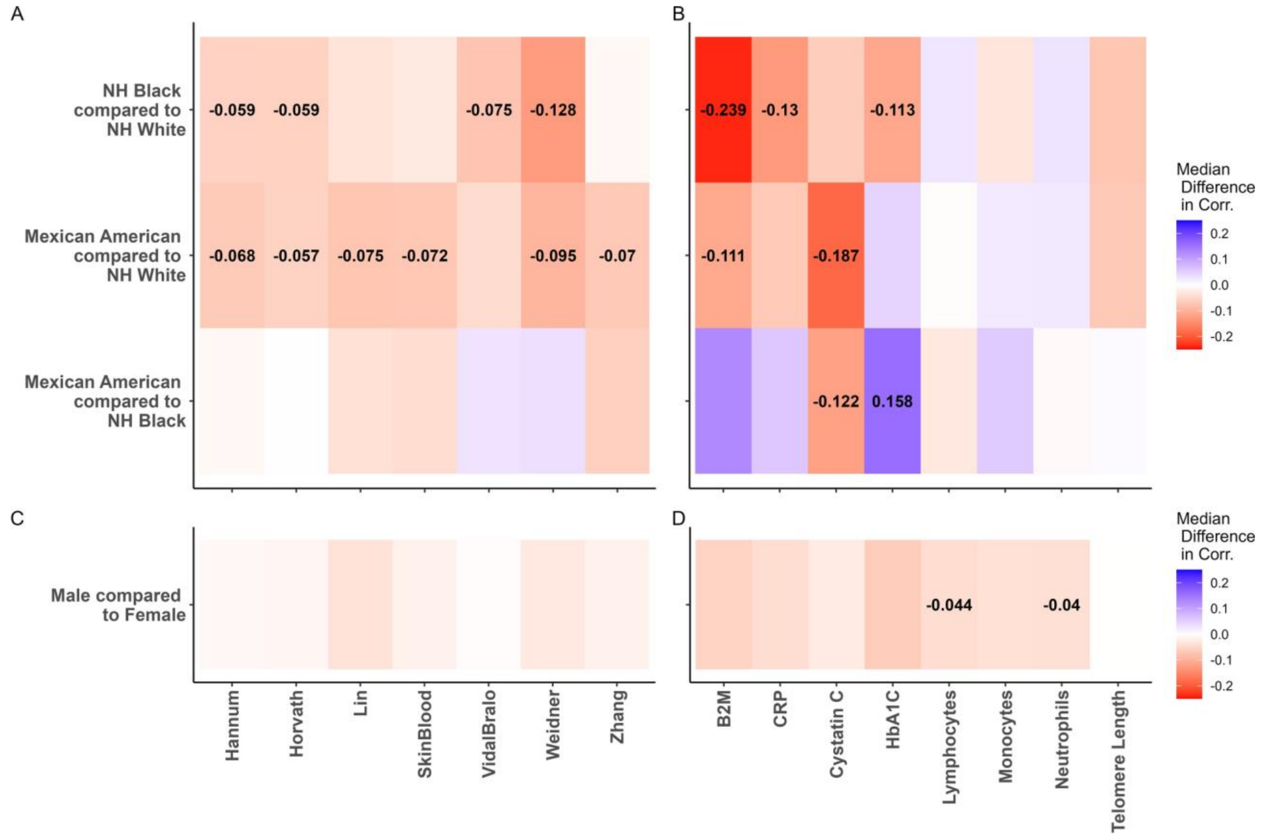


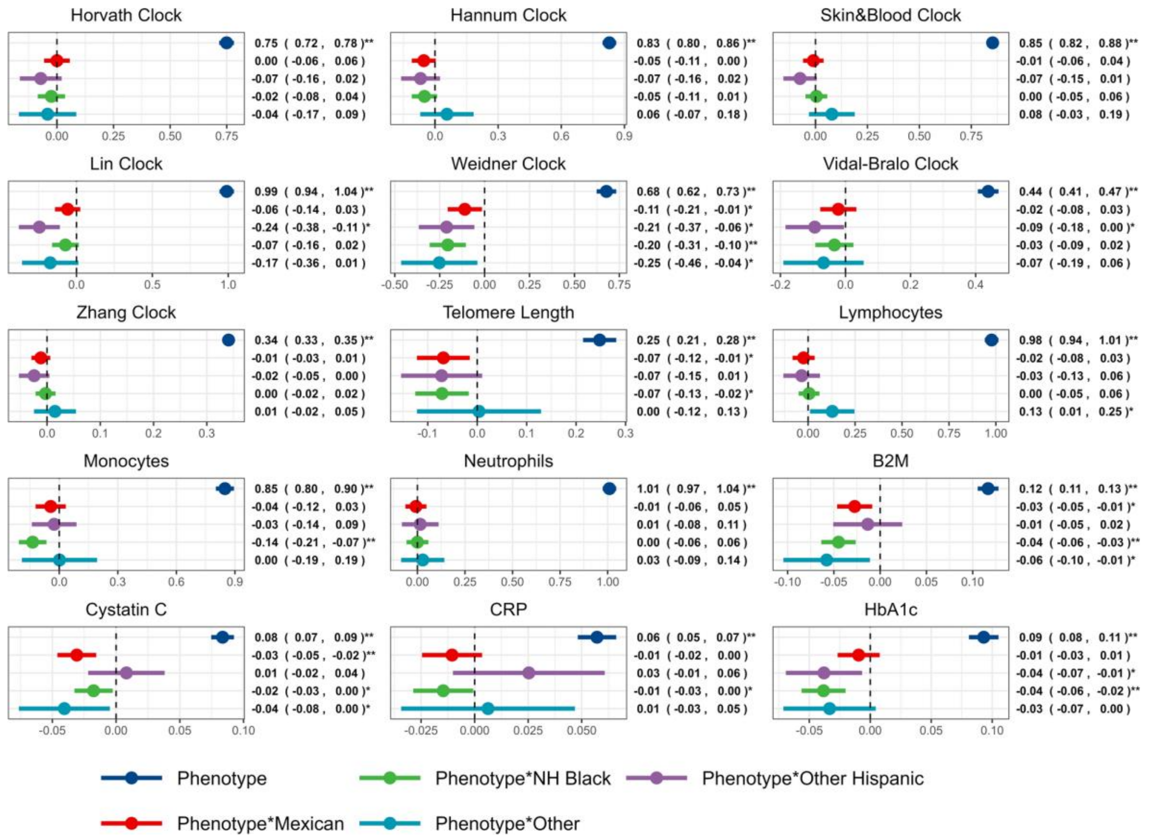


Teschendorff, AE., and S Horvath. *Nature Reviews Genetics* (2025): 1-19.

Epigenetic Biomarkers Performance

- Performance by race/ethnicity and sex among 2,532 U.S. Adults (NHANES 1999-2001)





*Adjusted for SES and cell-type composition

Khodasevich, D, et al. *Clinical Epigenetics* 17 (2025): 59.

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Other Epigenetic Biomarkers

Risk factor	Existing Epigenetic Biomarker (Methylation Risk Scores)
Smoking	EpiSmoker and *AHRR*
Alcohol intake	Alcohol Score
Age	Several epigenetic clocks
Lead Exposure	Bone Pb MRS
Inflammatory plasma proteins	*EpiScores*
Metabolomic	MRS Metabolomics

Aim 3- Smoking and Bone Lead

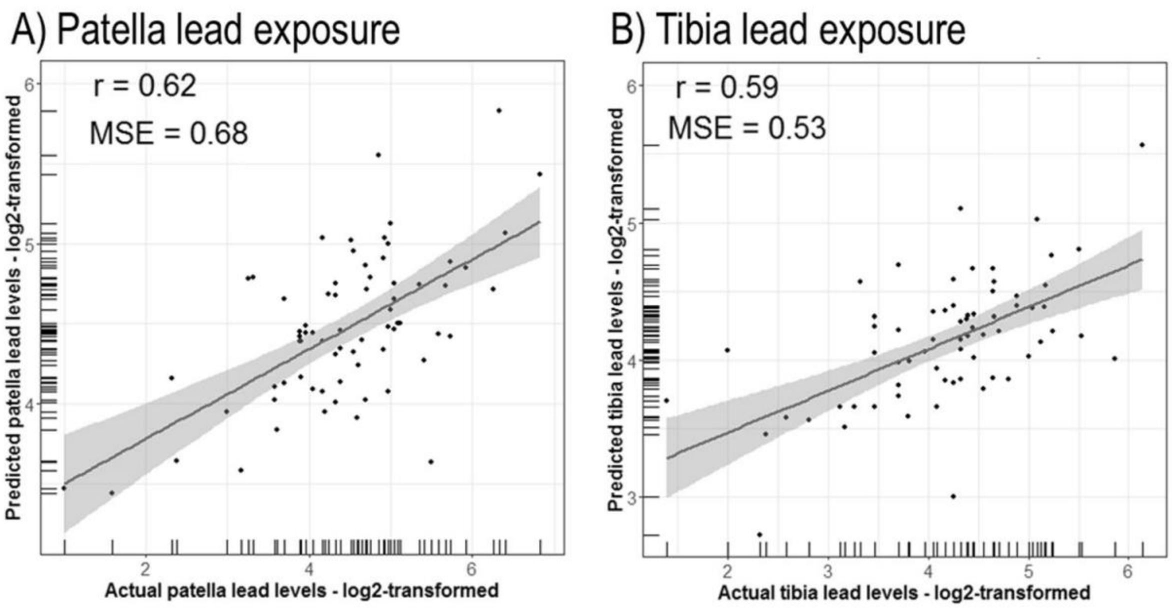
Smoking

Table 3. Prediction estimates from smoking status classifier, across the training and test datasets.

Datasets	Sensitivity (%)	Specificity (%)
Training dataset (DILGOM, N = 474):		
– Current vs others	75	98
– Former vs others	60	99
– Never vs others	99	72
Test datasets		
FTC (N = 408):		
– Current vs others	82	97
– Former vs others	22	96
– Never vs others	96	47
EIRA (N = 687):		
– Current vs others	69	84
– Former vs others	14	97
– Never vs others	95	58
CARDIOGENICS (N = 464):		
– Current vs others	91	73
– Former vs others	19	95
– Never vs others	92	65

Bollepalli, Sailalitha, et al. _Epigenomics_ 11.13 (2019): 1469-1486.

Bone lead



Colicino E, et al. JESEE (2019): 1-9.

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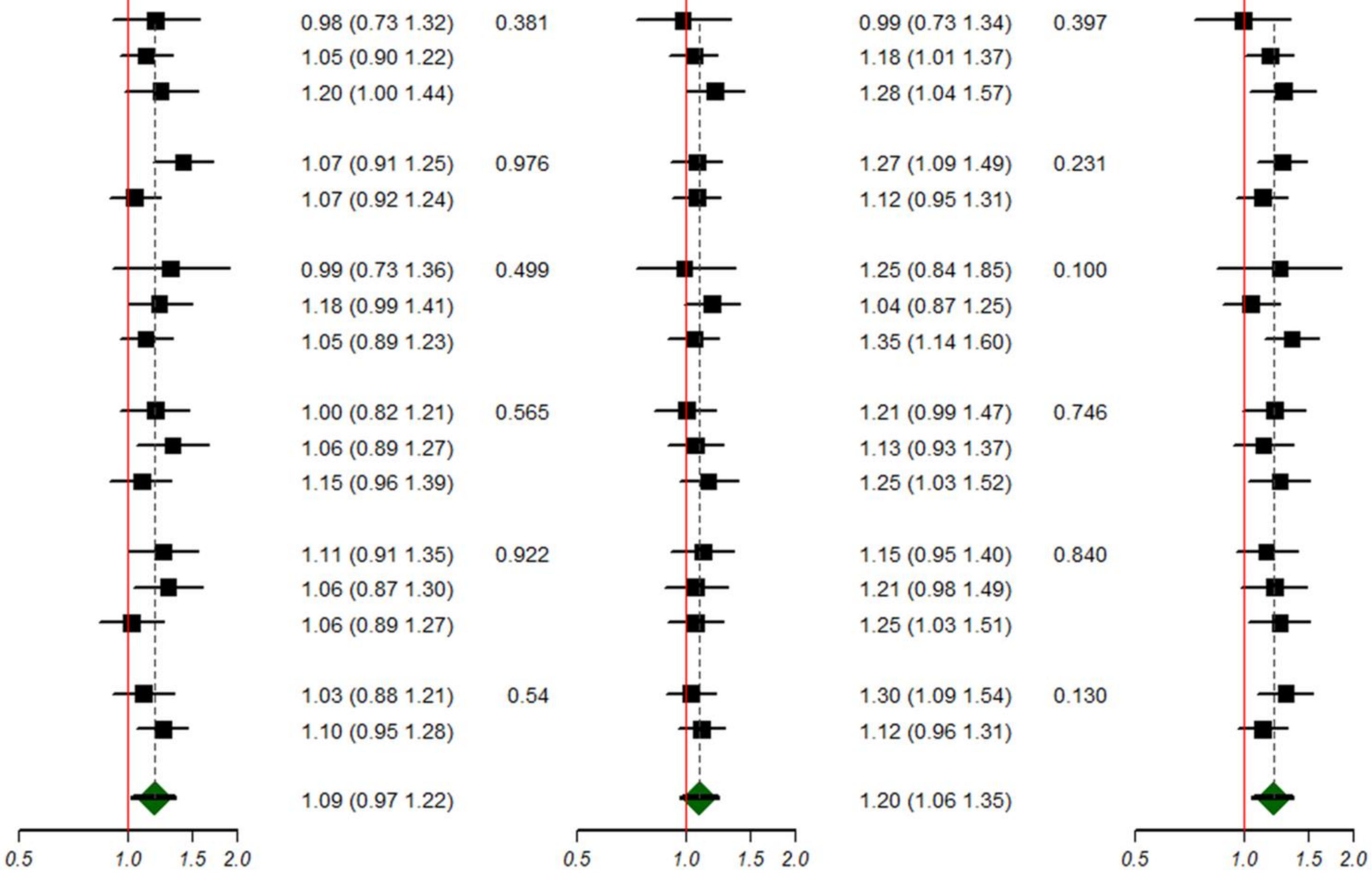
Bone Lead & CVD in SHS



Subgroup	Cases / Non Cases	A: HR (95% CI)	A: P for Interaction	B: HR (95% CI)	B: P for Interaction	C: HR (95% CI)	C: P for Interaction
Age (years)							
44.0-49.9	81 / 587	1.19 (0.91 1.58)	0.794	0.98 (0.73 1.32)	0.381	0.99 (0.73 1.34)	0.397
50.0-64.9	253 / 991	1.12 (0.95 1.33)		1.05 (0.90 1.22)		1.18 (1.01 1.37)	
65.0-75.4	118 / 291	1.23 (0.98 1.56)		1.20 (1.00 1.44)		1.28 (1.04 1.57)	
Sex							
Male	195 / 767	1.42 (1.17 1.72)	0.014	1.07 (0.91 1.25)	0.976	1.27 (1.09 1.49)	0.231
Female	257 / 1102	1.04 (0.89 1.23)		1.07 (0.92 1.24)		1.12 (0.95 1.31)	
Center							
AZ	47 / 264	1.31 (0.91 1.90)	0.661	0.99 (0.73 1.36)	0.499	1.25 (0.84 1.85)	0.100
OK	173 / 808	1.22 (1.00 1.50)		1.18 (0.99 1.41)		1.04 (0.87 1.25)	
ND/SD	232 / 797	1.12 (0.95 1.33)		1.05 (0.89 1.23)		1.35 (1.14 1.60)	
Smoking Status							
Never	141 / 543	1.19 (0.95 1.48)	0.391	1.00 (0.82 1.21)	0.565	1.21 (0.99 1.47)	0.746
Former	140 / 605	1.33 (1.06 1.66)		1.06 (0.89 1.27)		1.13 (0.93 1.37)	
Current	171 / 721	1.09 (0.89 1.32)		1.15 (0.96 1.39)		1.25 (1.03 1.52)	
Urine Cadmium (µg/g)							
<0.76	141 / 617	1.25 (1.00 1.56)	0.208	1.11 (0.91 1.35)	0.922	1.15 (0.95 1.40)	0.840
0.76-1.26	145 / 645	1.29 (1.04 1.61)		1.06 (0.87 1.30)		1.21 (0.98 1.49)	
>1.26	166 / 607	1.02 (0.83 1.25)		1.06 (0.89 1.27)		1.25 (1.03 1.51)	
Diabetes Status							
No	184 / 1171	1.10 (0.91 1.34)	0.322	1.03 (0.88 1.21)	0.54	1.30 (1.09 1.54)	0.130
Yes	268 / 698	1.25 (1.06 1.46)		1.10 (0.95 1.28)		1.12 (0.96 1.31)	
Overall	452 / 1869	1.18 (1.03 1.34)		1.09 (0.97 1.22)		1.20 (1.06 1.35)	

Lieberman-Cribbin, Wil, et al. *Journal of the American Heart Association* 11.23 (2022): e026934.

Stanford University

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Research Group

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UC BERKELEY SUPERFUND RESEARCH PROGRAM SCIENCE FOR A SAFER WORLD

NIH NIEHS

NIH NIA

NIH NHLBI

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The Nutrition and Physical Activity Assessment Study (NPAAS) research program 2004-present

Marian L. Neuhouser, PhD, RD
Professor and Program Head
Cancer Prevention Program
Division of Public Health Sciences
Fred Hutchinson Cancer Center

WHI Annual Investigator Meeting - May 1, 2025

20+ years of Nutritional Biomarker Studies in WHI

2004 — **Nutritional Biomarkers Study (NBS)**
DM Trial I and C (n=544)

2006 — **Nutrition and Physical Activity Assessment Study (NPAAS)**
OS (n=450)

2010 — **NPAAS II - Nutrition and Physical Activity Assessment Study Feeding Study**
NPAAS-FS DM-C, OS, HT (n=153)

2016 — **NPAAS III–Metabolomics and Statistical Methods**
NPAAS, NPAAS-FS, WHI BMD Breast & CRC & cases (n=1516)

2020 — **NPAAS IV - Calibration Methods, Biomarkers, Stable Isotopes, Metabolomics, Dietary Patterns, Replication in Diverse Populations**

2024 — **NPAAS V - Metabolomic Biomarkers of WHI-DM, Calibration of DM**
WHI DM/blood draw cohort/NBS (n=1000)

AS289-Pooling Project
International IAEA Collab
AS693-Dietary Metabolite Validation
M12-Urinary Sugars
AS417-Metabolomics
AS423-Stable Isotopes
AS525-Na, K, Creatinine
AS560-Food Metabolites

>90 manuscripts, 8 funded ancillary studies, 9 graduate students, 9 postdocs, other new collaborations

WHI Nutritional Biomarkers Study (NBS) 2004-2005: final year of WHI-DM*

The WHI FFQ was the principal adherence monitoring tool for the WHI DM

- Emerging evidence that most measures of dietary self-report had both random and systematic error
- We needed methods to properly interpret the ensuing WHI-DM results
- Recovery biomarkers where:
  - Intake = Output

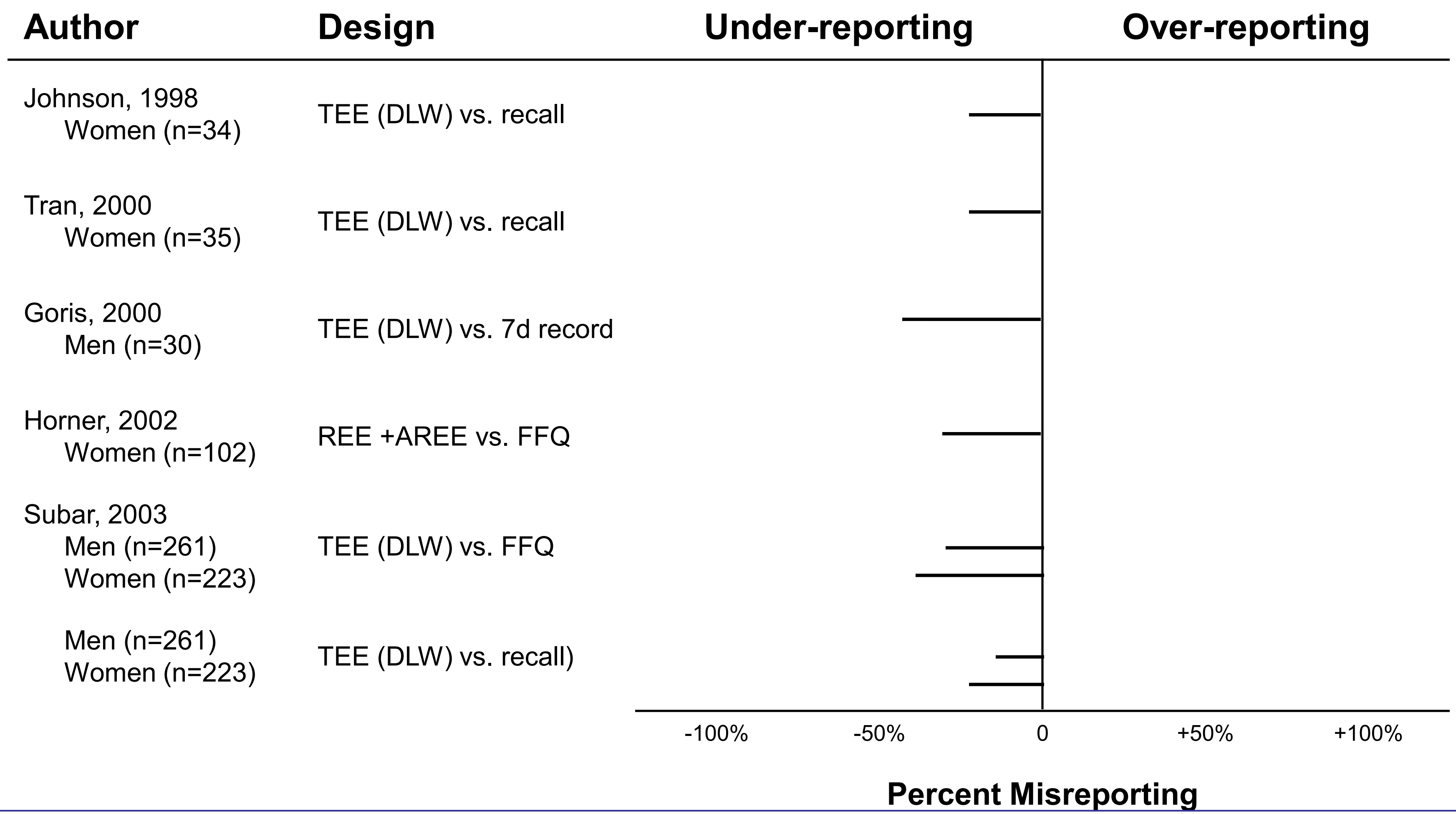
  → used as approach for understanding the phenomena

WHI Form 60: FFQ

TYPE OF FOOD	HOW OFTEN DID YOU EAT THE FOOD (Mark one)									AMOUNT			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving Size	Your Serving Size S	M	L
Orange juice and grapefruit juice										6 ounce glass			
Tang®, Kool-Aid®, Hi-C®, and other fruit drinks										6 ounce glass			
Other fruit juices such as apple, grape										6 ounce glass			
VEGETABLES													
Green or string beans										1/2 cup			
Green or English peas										1/2 cup			
Refried beans										3/4 cup			
All other beans such as baked beans, lima beans, black-eyed peas and chili without meat										3/4 cup			
Tofu and textured vegetable products										3 slices or 3 ounces			
Avocado and guacamole, including added to mixed dishes										1/4 medium or 1/4 cup			
Corn and hominy										1/2 cup			
Tomatoes, fresh or juice										1 medium or 6 ounce glass			
Tomatoes cooked, tomato sauce, salsa and salsa picante										1/2 cup			
Green peppers, green chilies, jajapeños, and green chili salsa										1/4 cup			
Red peppers and red chilies										1/4 cup			

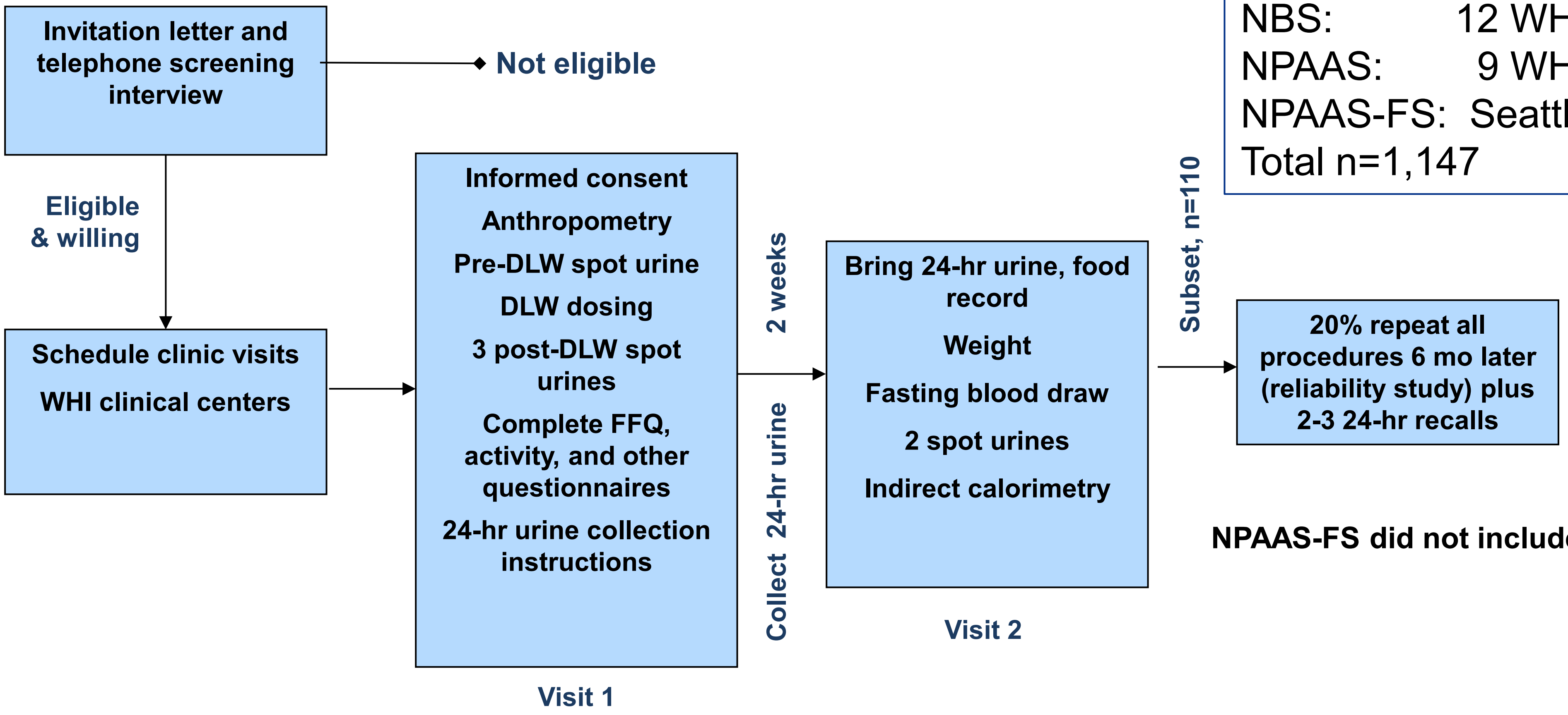
* 12 WHI CCs participated, CCC led and coordinated

Early studies of misreporting of nutrients using recovery biomarkers: energy (DLW=doubly labeled water*)



Energy intake ~ Energy expenditure (DLW)* in weight stable people. DLW 97% accurate vs. whole room calorimeter

Nutritional Biomarker Studies in the Women's Health Initiative - Design



NBS: 12 WHI CCs; WHI-DM
NPAAS: 9 WHI CCs; WHI-OS
NPAAS-FS: Seattle only; OS or CT
Total n=1,147

NPAAS-FS did not include reliability study

Urine biomarkers: DLW-TEE, nitrogen, sodium, potassium, sugars, metabolomics (NMR).
Blood biomarkers: vitamins, carotenoids, phospholipid fatty acids, carbon and nitrogen stable isotope ratios (subset), metabolomics (both aqueous LC/MS and lipids from Lipidyzer)

Early findings - discovery

WHI NBS assessments, mean (SD)	WHI-DM-I	WHI-DM-C
Self-report energy: FFQ kcal/d	1445 (504)	1647 (554)
Recovery biomarker: TEE kcal/d	2070 (340)	2086 (334)
Self-report protein: FFQ g/d	65 (24)	69 (26)
Recovery biomarker: protein g/d	75 (22)	73 (19)

Neuhouser et al *Am J Epidemiol* 2008

- Measurement error in the WHI FFQ was **systematic** and related to participant personal characteristics
  - Statistically significant **underreporting** for:
    - WHI DM intervention arm#, BMI**, Black*, Hispanic*
  - Statistically significant **overreporting** for:
    - Age*, other race/ethnicity*, current smoking§

#energy only
*energy *and* protein
**energy *and* %energy protein
§ %energy protein only

Methods development and application

Example of development of calibration equations and application to FFQ: energy

BMI category kg/m^2	Self report FFQ Geometric mean (IQR)	DLW-TEE Geometric mean (IQR)	Calibrated FFQ* Geometric mean (IQR)
<25.0	1407 (1157-1759)	1894 (1714-2083)	1912 (1853-1980)
25.0-29.9	1462 (1196-1837)	2043 (1904-2232)	2028 (1962-2103)
$\geq$ 30.0	1454 (1161-1897)	2213 (2034-2415)	2247 (2156-2338)
* Predicted values utilizing the objective biomarker and considers measurement error in self-report			

Neuhouser et al *Am J Epidemiol* 2008

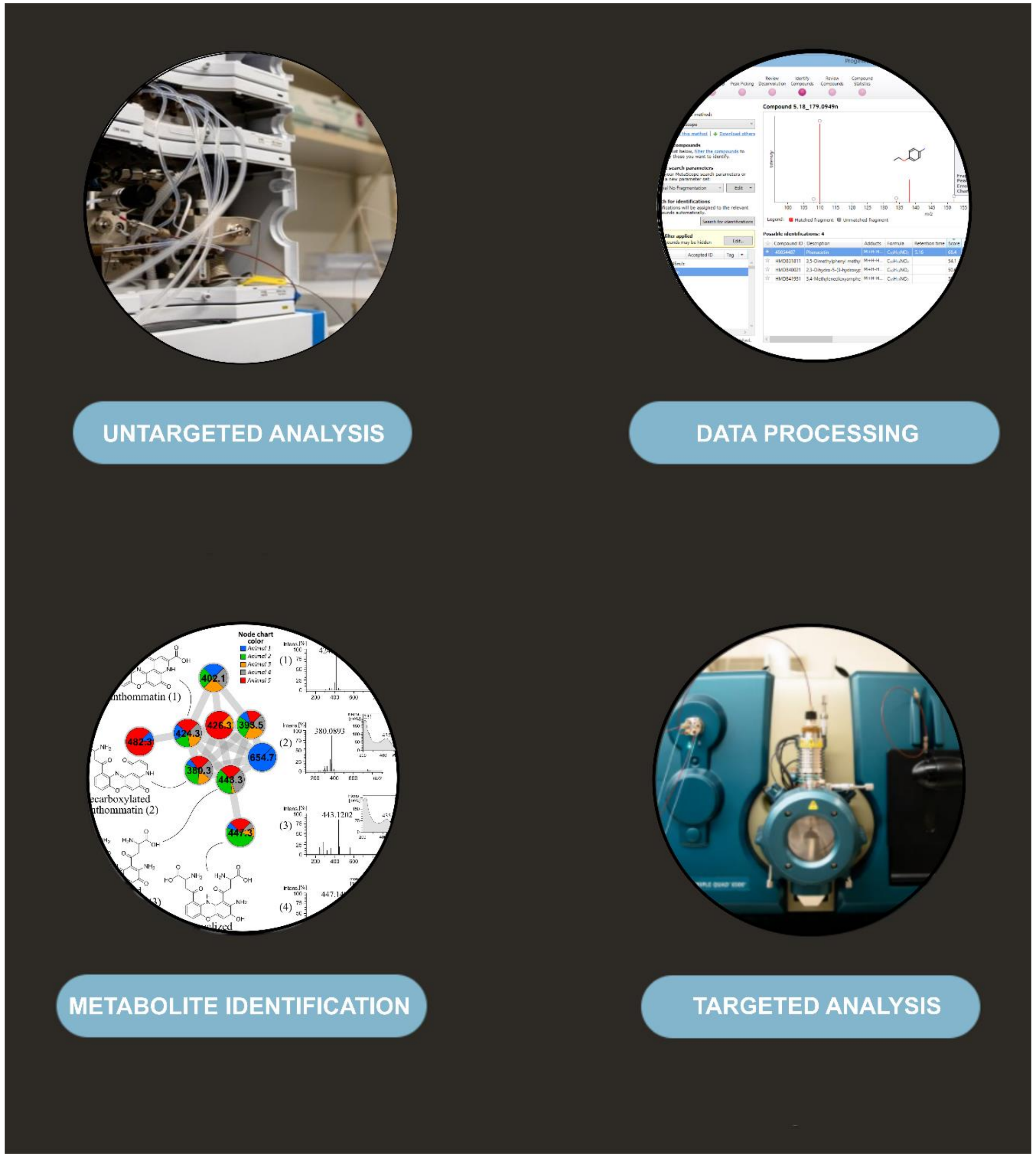
Metabolomics in NPAAS

- Nutrient-based recovery and concentration biomarkers may not sufficiently reflect the complexity of food intake or dietary patterns
- **Metabolomics** → comprehensive study of the metabolome
  small molecules in cells, tissues and bodily fluids
  aqueous and lipids
  useful in discovery of nutritional biomarkers; may reflect intake and metabolism;
  →mechanisms – tie to biochemical pathways

- Useful nutritional biomarkers (including metabolomics) should:
  - Adhere to Bradford Hill criteria:

    Biological plausibility, dose-response, time-course, effect size, reproducibility

    Landberg, *Nutrition Reviews*, 2023
    Dragstad, *Genes Nutr*, 2028
  - Have reliable food and nutrient database values



Metabolomics

UW Northwest Metabolomics Research Center

NPAAS has metabolite data on >1000 WHI participants using these platforms:

- Serum: LC-MS/MS (aqueous)
- Serum: Lipidyzer AB Sciex QTRAP (lipids)
- Urine: ^{1}NMR spectroscopy
- Urine: GC-MS

- All NPAAS data generated with NWMRC platforms except AS 560 (Metabolon)

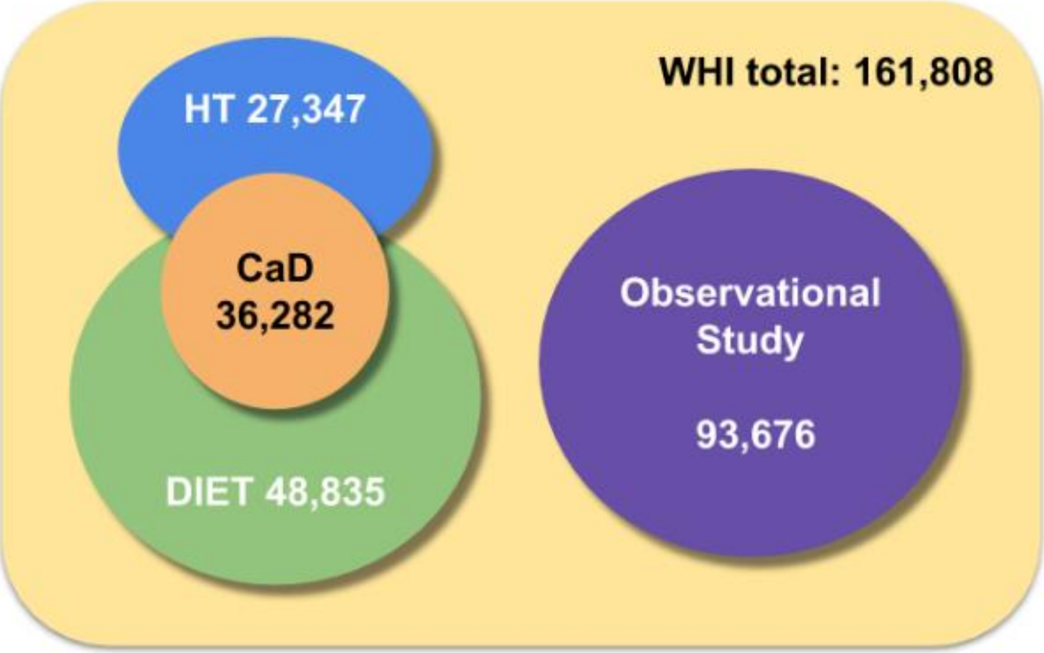
Phased approach to discovery and application

Metabolite discovery in NPAAS-FS

Calibration equation development in NPAAS-OS

Application of calibrated self-report to WHI cohorts

TYPE OF FOOD	HOW OFTEN DID YOU EAT THE FOOD (Mark one)									AMOUNT			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving Size	Your Serving Size S	M	L
Orange juice and grapefruit juice										6 ounce glass			
Tang®, Kool-Aid®, Hi-C®, and other fruit drinks										6 ounce glass			
Other fruit juices such as apple, grape										6 ounce glass			
VEGETABLES													
Green or string beans										1/2 cup			
Green or English peas										1/2 cup			
Refried beans										3/4 cup			
All other beans such as baked beans, lima beans, black-eyed peas and chili without meat										3/4 cup			
Tofu and textured vegetable products										3 slices or 3 ounces			
Avocado and guacamole, including added to mixed dishes										1/4 medium or 1/4 cup			
Corn and hominy										1/2 cup			
Tomatoes, fresh or juice										1 medium or 6 ounce glass			
Tomatoes cooked, tomato sauce, salsa and salsa picante										1/2 cup			
Green peppers, green chilies, jajapeños, and green chili salsa										1/4 cup			
Red peppers and red chilies										1/4 cup			



Example of discovery and application using metabolomics

Biomarker-Calibrated Red and Combined Red and Processed Meat Intakes with Chronic Disease Risk in a Cohort of Postmenopausal Women

Cheng Zheng,[1] Mary Pettinger,[2] GA Nagana Gowda,[3] Johanna W Lampe,[2,4] Daniel Raftery,[3] Lesley F Tinker,[2] Ying Huang,[2,4] Sandi L Navarro,[2] Diane M O'Brien,[5] Linda Snetselaar,[6] Simin Liu,[7] Robert B Wallace,[6] Marian L Neuhouser,[2,4] and Ross L Prentice[2,4]

J Nutr 2022

Metabolites and Variables for Red + Processed Meat (NPAAS-FS)

Variable	Coefficient	R^2	CV-R^2
(Intercept)	-224.7		
Creatine (urine)	13.5	9.2%	7.6%
Trimethylamine (urine)	25.4	0.3%	0.2%
Trimethylamine.N.oxide (urine)	-10.4	6.9%	5.7%
Guanidinoacetate (urine)	-47.5	5.2%	4.3%
Acetylcarnitine (serum)	13.9	3.2%	2.6%
Hydroxyproline (serum)	24.2	5.8%	4.8%
Biliverdin (serum)	-5.1	1.6%	1.3%
Lysophosphatidylcholine (LPC 22:5) (serum)	12.6	2.5%	2.0%
Phosphatidylcholine (PC 38:0) (serum)	-8.8	3.0%	2.5%
Phosphatidylcholine (PC 38:4) (serum)	14.4	0.6%	0.5%
BMI (kg/m^2)	1.5	3.3%	2.7%
Urinary nitrogen	33.1	3.8%	3.1%
Baseline FFQ Total meat (g/d)	0.2	9.5%	7.8%
TOTAL		**54.9%**	**45.0%**

Cancer outcomes in the WHI

	With Biomarker Calibration				Without Biomarker Calibration			
	Red Meat Intake (g/d)		Red + Processed Meat Intake (g/d)		Red Meat Intake (g/d)		Red + Processed Meat Intake (g/d)	
Cancer Site (n cases)	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Breast (5139)	**1.10 (1.07, 1.13)**	<0.001	**1.09 (1.07, 1.12)**	<0.001	1.02 (1.01, 1.03)	0.001	1.02 (1.01, 1.04)	0.001
Colon (1060)	**1.12 (1.06, 1.18)**	0.0001	**1.11 (1.05, 1.16)**	<0.001	1.03 (1.00, 1.05)	0.06	1.03 (1.00, 1.06)	0.06
Rectum (158)	1.01 (0.86, 1.17)	0.94	1.02 (0.89, 1.17)	0.78	1.00 (0.93, 1.07)	>0.99	1.01 (0.93, 1.09)	0.86
Endometrium (881)	**1.25 (1.18, 1.33)**	<0.001	**1.24 (1.18, 1.31)**	<0.001	1.01 (0.98, 1.04)	0.58	1.01 (0.98, 1.04)	0.51
Obesity-related (7313)	**1.12 (1.09, 1.14)**	<0.001	**1.11 (1.09, 1.13)**	<0.001	1.02 (1.01, 1.03)	0.001	1.02 (1.01, 1.03)	<0.001
Total Invasive (12,804)	**1.07 (1.05, 1.09)**	<0.001	**1.07 (1.05, 1.08)**	<0.001	1.01 (1.00, 1.02)	0.01	1.01 (1.00, 1.02)	0.003

Next steps and how to get involved

- More metabolomics data are being generated
- Data to date can be shared with approved WHI manuscript proposal
  - not part of WHI investigator dataset
- Limited NPAAS-OS and NPASS-FS biospecimens remain; use requires approved WHI ancillary study
- Interested? Reach out to:
  - Marian Neuhouser (mneuhous@fredhutch.org) or
  - Johanna Lampe (jlampe@fredhutch.org)

Acknowledgments

Plus

WHI participants

WHI CCC staff

Sheri Greaves

Jen Bryce

Todd Panek

Mary Pettinger (retired)

Grad students

Postdocs

Many others!

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Recent Efforts to Bring Objective Dietary Measures into Nutritional Epidemiology Studies in WHI Cohorts

Energy intake assessment

Macronutrient composition of the diet and energy intake

Cohort/case-control studies of dietary composition and chronic disease risk in WHI cohorts

Total energy intake biomarker, and total mortality association

Prentice et al (2024, AJCN)

- **Total Energy Expenditure** (TEE) assessed using doubly-labeled water (DLW)
- Comparison with TEE reveals **major systematic biases in self-reported total energy assessment** whether using FFQs, 4-day FRs, or 3 three 24HRs (Neuhouser et al, 2008, AJE; Prentice et al, 2011, AJE; Freedman et al, 2014, AJE)
- Linear regression of **log-feeding study energy intake** on log- TEE and log-weight variation (i.e. weight at end/ weight at start) of 2-week DLW protocol period in NPAAS-FS (n= 153)
- **log EI = 2.622 + 0.661 log TEE + 5.192 log weight variation**
- Correlation of log EI with feeding study log energy intake of **0.73**
- **Total mortality log HR** modeled as a linear function of **log EI** as well as potential interactive and confounding factors (n=1,131)

Macronutrient composition of the diet and total energy intake

- Metabolomic-based biomarkers for macronutrient/macronutrient component densities (g/kcal), without any use of self-reported dietary data:

- Carbohydrate (added sugars, fiber)
- Protein (animal protein)
- Saturated, polyunsaturated, and monounsaturated fatty acid

[Prentice et al (AJCN, 2025)]

Biomarker equation for log-carbohydrate density (n=153)

Regression Variables (each log-transformed)	Beta	R^2	CV-R^2
(Intercept)	-3.51932		
Phosphatidylcholine (PC 18:1, 22:5) (serum)	0.135792	23.20%	**17.60%**
Urinary nitrogen	-0.13399	1.60%	1.20%
Sucrose (urine)	0.076076	8.10%	**6.10%**
Triacylglycerol (TAG 50:4, FA18:0) (serum)	0.11047	10.40%	**7.90%**
Total energy expenditure (TEE)	0.2221	4.00%	3.00%
Triacylglycerol (TAG 52:4, FA20:2) (serum)	0.11291	1.30%	1.00%
Phosphatidylcholine (PC 18:0, 22:5) (serum)	0.092417	1.40%	1.10%
Maltose (urine)	0.0169	1.20%	0.90%
Lysophosphatidylcholine (LPC 22:5) (serum)	0.029806	0.40%	0.30%
Total		51.50%	39.10%

Biomarker equation for log-protein density (n=153)

Regression Variables (each log transformed)	Beta	R^2	CV-R^2
(Intercept)	-2.95298		
Urinary nitrogen	0.338921	20.70%	**12.00%**
3-Hydroxyisovaleric acid (urine)	-0.16989	14.20%	**8.20%**
Lysophosphatidylethanolamine (LPE 16:0*) (serum)	0.208009	6.80%	**3.90%**
Total energy expenditure (TEE)	-0.20638	3.10%	1.80%
Creatine (serum)	0.073162	8.80%	**5.10%**
Methyl glycocholate (urine)	-0.03517	2.60%	1.50%
2-Hydroxybutyrate (serum)	0.06946	4.00%	2.30%
Maltose (urine)	-0.01628	1.20%	0.70%
Weight at end/weight at start of DLW protocol period	-1.21879	0.50%	0.30%
2-Oxoisovalerate (serum)	0.052842	1.20%	0.70%
Cortisol (serum)	0.034668	0.50%	0.30%
1/3-Methylhistidine (serum)	0.011928	0.40%	0.30%
Propanediol (urine)	-0.02355	0.30%	0.20%
Lysophosphatidylcholine(LPC 18:1*)(serum)	-0.0829	0.30%	0.20%
Cholesteryl ester (CE 22:6*) (serum)	0.028684	0.20%	0.10%
Total		64.70%	37.50%

Association of biomarker energy intake with carbohydrate- and protein- related densities (n=368)

Model: Linear regression of *log biomarker EI* on *log macronutrient density* variables	Source	Coeff	SE	P-value	R^2-term	R^2-total
Macronutrient density variable						
Carbohydrate	Biomarker	**0.107**	0.053	**0.045**	0.8%	31.0%
Protein	Biomarker	**-0.117**	0.045	**0.009**	1.3%	
Carbohydrate	4DFR	-0.034	0.036	0.35	0.2%	29.3%
Protein	4DFR	**-0.080**	0.030	**0.008**	1.4%	
Carbohydrate	24HRs	-0.035	0.036	0.34	0.2%	28.9%
Protein	24HRs	**-0.067**	0.031	**0.030**	1.0%	
Carbohydrate	FFQ	**-0.074**	0.034	**0.030**	0.9%	29.4%
Protein	FFQ	**-0.062**	0.031	**0.042**	0.8%	

Association of **log-energy intake** with **log-fatty acid densities** (NPAAS)

	Source	Coeff	SE	P-value	R^2-term	R^2-total
Fatty Acids Category						
Saturated	**Biomarker**	**0.073**	**0.043**	**0.094**	**0.6%**	**29.7%**
Polyunsaturated	**Biomarker**	**0.079**	**0.032**	**0.014**	**1.2%**	
Monounsaturated	**Biomarker**	**-0.085**	**0.040**	**0.037**	**0.9%**	
Saturated	*4DFR*	*0.040*	*0.028*	*0.15*	*0.4%*	*28.5%*
Polyunsaturated	*4DFR*	*-0.002*	*0.022*	*0.94*	*<0.1%*	
Monounsaturated	*4DFR*	*-0.019*	*0.037*	*0.61*	*0.1%*	
Saturated	**24HRs**	**0.031**	**0.025**	**0.21**	**0.3%**	**28.4%**
Polyunsaturated	**24HRs**	**-0.003**	**0.020**	**0.88**	**<0.1%**	
Monounsaturated	**24HRs**	**-0.006**	**0.033**	**0.85**	**<0.1%**	
Saturated	*FFQ*	*0.025*	*0.032*	*0.43*	*0.1%*	*29.1%*
Polyunsaturated	*FFQ*	*-0.036*	*0.036*	*0.31*	*0.2%*	
Monounsaturated	*FFQ*	*0.059*	*0.052*	*0.27*	*0.3%*	

Table 2. Linear regression CV-R^2 values for biomarker equations for **dietary** log-transformed fatty acid densities and related composite density variables (NPAAS-FS)

Density Variable	CV-R^2 (%)	Density Variable	CV-R^2 (%)	Density Variable	CV-R^2(%)
SFA (common name)		MUFA (common name)		Composite FAs	
4:0 (butyric)	**64.7**	14:1 (myristoleic)	4.5	SFA total[1]	**46.4**
6:0 (caproic)	**60.9**	16:1 (palmitoleic)	21.3	MUFA total[1]	29.9
8:0 (caprylic)	**48.7**	18:1 (oleic)	31.3	PUFA total[1]	52.8
10:0 (capric)	**53.0**	20:1 (eicosenoic)	22.8	Omega 3 (n-3) PUFA	**46.1**
12:0 (lauric)	39.9	22:1 (erucic)	23.4	Omega 6 (n-6) PUFA	**52.4**
14:0 (myristic)	**61.0**	PUFA (common name)		Macronutrients	
16:0 (palmitic)	**42.2**	18:2 (linoleic)	**51.7**	Total fat	12.4
17:0 (heptadecanoic)	28.4	18:3 (alpha linolenic)	**50.1**	Total carbohydrates	38.4
18:0 (stearic)	**34.2**	18:3 (gamma linolenic)	24.5	Total protein	37.9
20:0 (arachidic)	34.8	20:4 (arachidonic)	39.7		
22:0 (decosanoic)	**49.9**	20:5 (eicosapentanoic-EPA)	**40.2**		
		22:5 (docosapentanoic-DPA)	**53.5**		
		22:6 (docosohexanoic-DHA)	**47.9**		

Summary /Future Research Opportunities

- **Self-reported EI** is not adequate for nutritional epidemiology purposes, whether using food records, recalls or frequencies.
- **Self-reported macronutrient component densities** may not be adequate for determining **dietary composition associations with EI**, with implications for obesity and chronic disease prevention research.
- Additional metabolomics-based **biomarker development research** is needed, preferably using a **habitual-diet feeding study design** (e.g. Prentice, Metabolites 2024).
- **Cohort/case-control studies** of key diet and disease associations with biomarker intake assessments are needed for a **fresh look at dietary composition and chronic disease associations broadly.**

[Breast and colorectal cancer case-control studies in 'bone centers' completed with biomarker-based macronutrient analyses underway]

End of Day 1

- Poster session and light refreshments: 4:15-5:30
  - Please join!
- Group dinner and celebration at Waterways: Doors open at 5:45
  - Across the street 901 Fairview Ave North Suite A-120
  - Pre-registration required
- ***Tomorrow morning – Friday***
  - 2K South Lake Union Walk – led by CCC staff
    - Meet in Silver Cloud Lobby at 7 am
- Meeting will be in the “Steam Plant” building on Friday
  - See meeting book for directions; walkway with steps is adjacent to Silver Cloud
  - CCC staff will be at entrance to let you into the building
  - Store luggage at Silver Cloud