Established and New Biomarkers in Nutritional Epidemiology

Ongoing efforts toward novel nutrient biomarker development

Ross Prentice

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Outline

- Nutrition and Physical Activity Assessment Study (NPAAS)
- Chronic disease association results using energy consumption and activity-related energy expenditure biomarkers
- Blood concentration measurements in the NPAAS-feeding study
- Metabolomic platforms for biomarker identification
- Recent efforts toward macronutrient biomarker identification

Nutrient and Physical Activity Assessment Studies (NPAAS) in WHI

- 544 DM Trial women completed two-week DLW protocol with urine and blood collection and FFQ (50% intervention, 50% control). A 20% reliability subsample repeated protocol. (NBS; 2004-2006)
- Biomarker study among 450 women in the OS for evaluating measurement properties of dietary and physical activity assessment approaches (frequencies, records, and recalls). With 20% reliability subsample. (NPAAS I; 2007-2009)
- Recently completed feeding study among 153 WHI women in Seattle, for development of objective markers for additional nutrients or foods. (NPAAS II; 2010-present)

Nutrition and Physical Activity Biomarker Study Collaborators (NBS and NPAAS)

Coordination (FHCRC):

Marian Neuhouser, Lesley Tinker, Johanna Lampe, Ross Prentice

Clinical Center PIs:

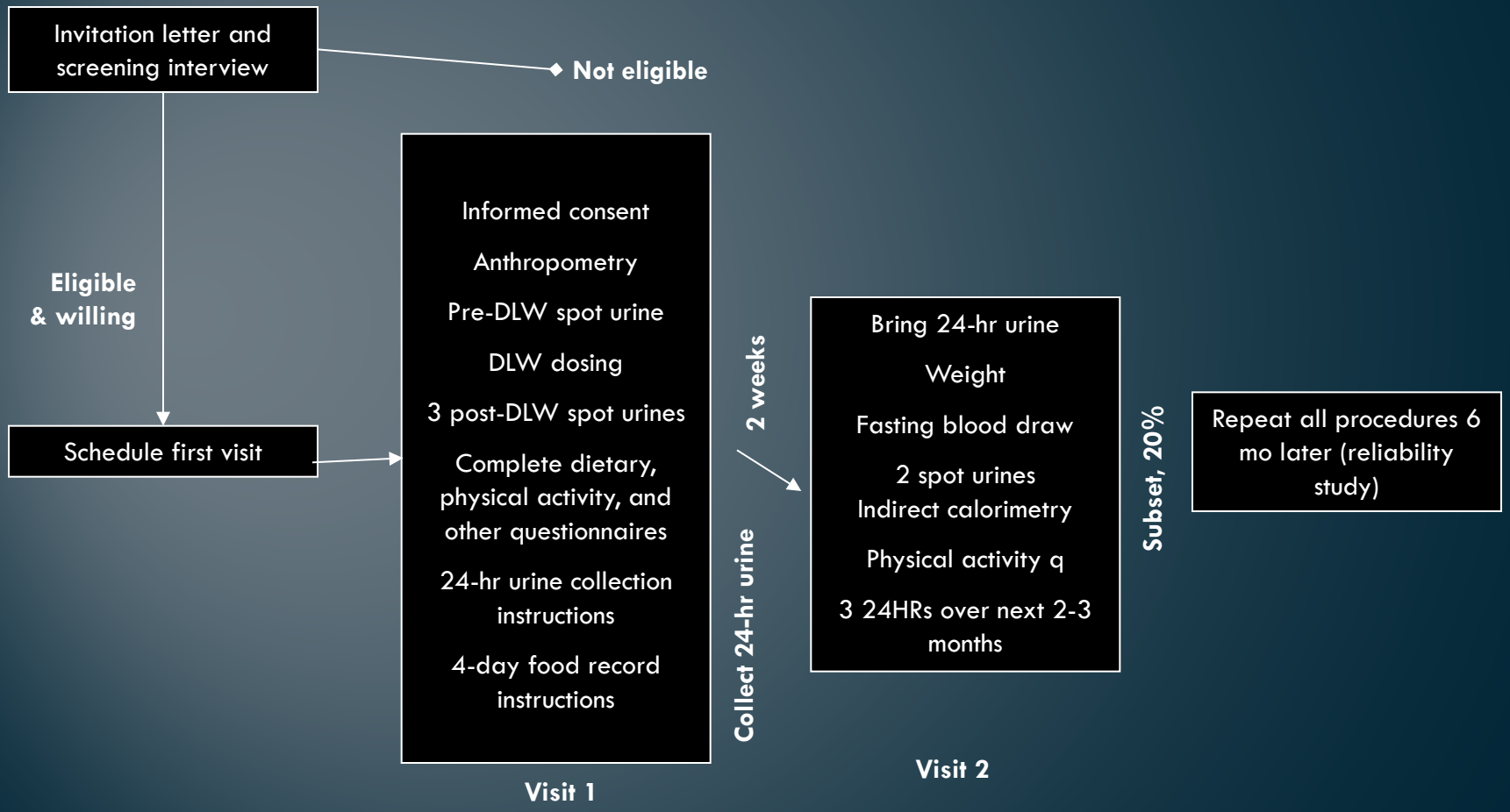
Shirley Beresford - Seattle
Bette Caan - Oakland
Linda Van Horn - Chicago
Cynthia Thomson - Arizona
Yasmin Mossavar-Rahmani† - NYC
Karen Johnson - Memphis
Gloria Sarto - Wisconsin
Judy Ockene - U Mass
Gerardo Heiss - UNC
Lewis Kuller* - Pittsburgh
Marcia Stefanick* - Stanford
Ellen Smit* - Portland
Annlouise Assaf* - Pawtucket

**NBS only †NPAAS only*

Additional Collaborators:

Dale Schoeller - Wisconsin (DLW); Sheila Bingham - Cambridge(UN); Xiaoling Song - Ying Huang, Chongzhi Di - FHCRC, Cheng Zheng - U of Wisconsin, Milwaukee; Pamela Shaw - U Penn; Jeannette Beasley – NYC; Dan Raftery - U of Washington

WHI Nutritional Biomarkers Study Procedures (NBS, NPAAS and NPAAS-FS)



Calibration Equation Coefficients (β), Standard Errors (SE), and Percent of Biomarker Variation Explained (R^2) from Regression of Log(biomarker) on Log(self-report), and Other Factors among 450 Observational Study Women

Energy

Variable	Food Frequency				4DFR				24HR			
	β	SE	R^2	Adj R^2	β	SE	R^2	Adj R^2	β	SE	R^2	Adj R^2
						ENERGY						
Intercept	7.614[a]	0.009			7.597[a]	0.009			7.607[a]	0.009		
FFQ	0.054[a]	0.017	3.8	6.5								
4DFR					0.161[a]	0.028	7.8	13.3				
24HR									0.101[a]	0.026	2.8	4.8
BMI	0.013[a]	0.001	26.9	45.9	0.013[a]	0.001	27.0	46.0	0.013[a]	0.001	28.7	48.9
Age	-0.010[a]	0.001	9.7	16.5	-0.009[a]	0.001	8.4	14.3	-0.009[a]	0.001	9.1	15.5
Black	-0.023	0.019			-0.024	0.018			-0.024	0.018		
Hispanic	-0.062[a]	0.021	1.3	2.2	-0.065[a]	0.020	1.5	2.6	-0.063[a]	0.020	1.5	2.6
Other minority	-0.041	0.040			-0.039	0.038			-0.038	0.039		
(Total)[b]			41.7	71.1			44.7	76.2			42.1	71.8

Prentice et al (2011, AJE)

Objective Measure of Physical Activity for Calibration of Self-Report Data

- Activity-Related Energy Expenditure (AREE)
  (Neuhouser et al, 2013, AJE)
- Energy and AREE in relation to cardiovascular disease, cancer and diabetes
  (Zheng et al, 2014, AJE)

Estimated Hazard Ratios for 20% Increments in Total Energy (TE) and in Activity-related Energy Expenditure (AREE), With and Without Calibration to Correct for Measurement Error, for Cardiovascular Diseases in the OS from Baseline (1994-1998) Through September 30, 2010

(Zheng et al, 2014, AJE)

	Uncalibrated				Calibrated			
	Energy		AREE		Energy		AREE	
Disease Category	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Total CHD	1.00	0.98,1.02	0.99	0.97,1.01	**1.57**	1.19,2.06	**0.78**	0.65,0.95
Nonfatal MI	1.00	0.98,1.03	0.99	0.97,1.01	1.49	1.13,1.97	0.80	0.67,0.97
Coronary Death	0.97	0.94,1.02	0.97	0.94,1.00	2.22	1.36,3.61	0.63	0.46,0.86
Heart Failure	1.04	1.01,1.08	0.97	0.95,1.00	**3.51**	2.12,5.82	**0.57**	0.41,0.79
CABG and PCI	1.01	0.99,1.03	1.01	0.99,1.03	1.43	1.19,1.70	0.90	0.79,1.03
Total Stroke	0.97	0.95,1.00	0.99	0.98,1.01	1.36	1.05,1.76	0.83	0.69,0.99
Ischemic Stroke	0.98	0.96,1.01	0.99	0.97,1.01	1.55	1.14,2.10	0.78	0.64,0.94
Hemorrhagic Stroke	0.94	0.88,0.99	1.03	0.99,1.08	0.47	0.21,1.07	1.37	0.85,2.20
Total CVD: CHD and Stroke	0.99	0.97,1.00	0.99	0.98,1.00	**1.49**	1.18,1.88	**0.80**	0.69,0.92
Total CVD including CABG and PCI	1.00	0.99,1.01	1.00	0.99,1.01	**1.49**	1.23,1.81	**0.83**	0.73,0.93

Estimated Hazard Ratio for 20% Increments in Total Energy (TE) and in Activity-related Energy Expenditure (AREE), With and Without Calibration to Correct for Measurement Error for Cancer, in the OS from baseline (1994-1998) Through September 30, 2010

(Zheng et al, 2014, AJE)

	Uncalibrated				Calibrated			
	Energy		AREE		Energy		AREE	
Cancer Category	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Total Invasive Cancer	1.01	1.00,1.02	0.99	0.99,1.00	**1.43**	1.17,1.73	**0.84**	0.73,0.96
Obesity-related Cancer	1.02	1.00,1.03	1.00	0.99,1.01	**1.71**	1.33,2.21	**0.79**	0.65,0.94
Breast Cancer	1.01	0.99,1.02	1.00	0.99,1.01	**1.47**	1.18,1.84	**0.82**	0.71,0.96
Colon Cancer	1.00	0.96,1.03	1.00	0.97,1.03	**1.86**	1.18,2.93	0.83	0.66,1.04
Rectum Cancer	1.01	0.92,1.10	0.99	0.93,1.05	**2.75**	1.10,6.83	**0.51**	0.27,0.99
Ovary Cancer	1.00	0.95,1.05	1.01	0.98,1.05	0.85	0.43,1.68	1.12	0.73,1.71
Endometrial Cancer	1.08	1.04,1.12	1.01	0.98,1.05	**2.72**	1.44,5.13	0.77	0.49,1.21
Bladder Cancer	1.03	0.97,1.10	0.96	0.92,1.00	1.80	0.88,3.69	0.68	0.42,1.09
Kidney Cancer	1.05	0.98,1.12	1.02	0.96,1.07	**2.94**	1.37,6.28	0.62	0.35,1.12
Pancreas Cancer	0.95	0.89,1.01	0.97	0.92,1.01	2.06	0.98,4.33	**0.61**	0.37,1.00
Lung Cancer	0.99	0.96,1.01	0.97	0.95,1.00	1.14	0.74,1.76	0.79	0.60,1.03
Lymphoma	1.08	1.03,1.13	1.00	0.96,1.03	0.99	0.48,2.07	1.16	0.69,1.94
Leukemia	1.01	0.95,1.07	0.98	0.93,1.02	1.48	0.70,3.12	0.74	0.47,1.18

Estimated Hazard Ratio for 20% Increments in Total Energy (TE) Consumption and in Activity-related Energy Expenditure (AREE), With and Without Calibration to Correct for Measurement Error, for Diabetes Incidence, in OS, from Baseline (1994-1998) Through September 17, 2012

(Zheng et al, 2014, AJE)

	Uncalibrated				Calibrated			
	Energy		AREE		Energy		AREE	
Outcome Category	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Diabetes Mellitus	1.06	1.04,1.07	1.01	1.00,1.02	**4.17**	2.68,6.49	**0.60**	0.44,0.83

Feeding study correlations (consumed vs. biomarker; Lampe et al 2016)

Benchmarks:

- Total energy (DLW) - 0.72
- Protein (UN) - 0.66

Serum Measures:

- Folate - 0.67
- Vit B12 - 0.71
- Alpha carotene - 0.73
- Beta-carotene - 0.61
- Lutein+zeaxanthin - 0.68
- Lycopene - 0.57
- Alpha tocopherol - 0.69
- % energy from MUFA - 0.65

Metabolomics as an Agnostic Approach to Novel Biomarker Development

Metabolomic platforms in blood and urine using NPAAS-FS specimens

Dan Raftery
University of Washington

Major Metabolomics Platforms

NMR

amino acids
organic acids
some amines
glucose
lipid classes

Detected molecules:
30-75

LC-MS

amino acids
amines
fatty acids
nucleosides
lipids
carbohydrates…

Detected molecules:
~1000 (300 ID'd)

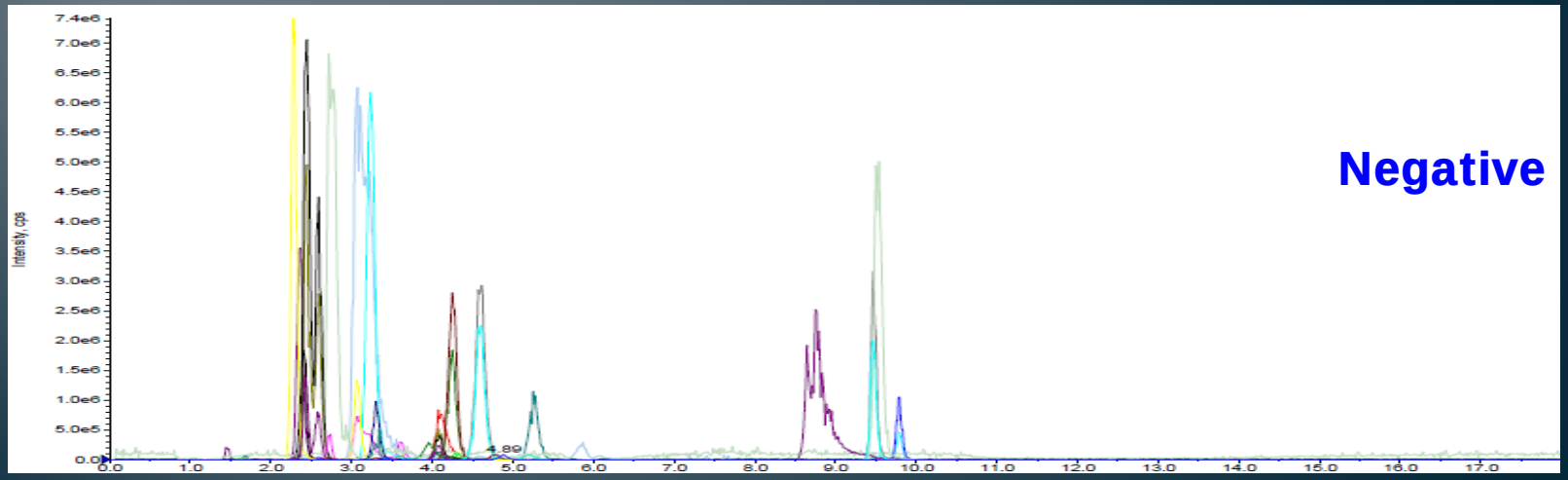
GC-MS

organic acids
aldehydes, ketones
other volatiles
fatty acids
amino acids
steroids

Detected molecules:
~200 (50-100 ID'd)

Serum Profiling: Targeted LC-MS Analysis

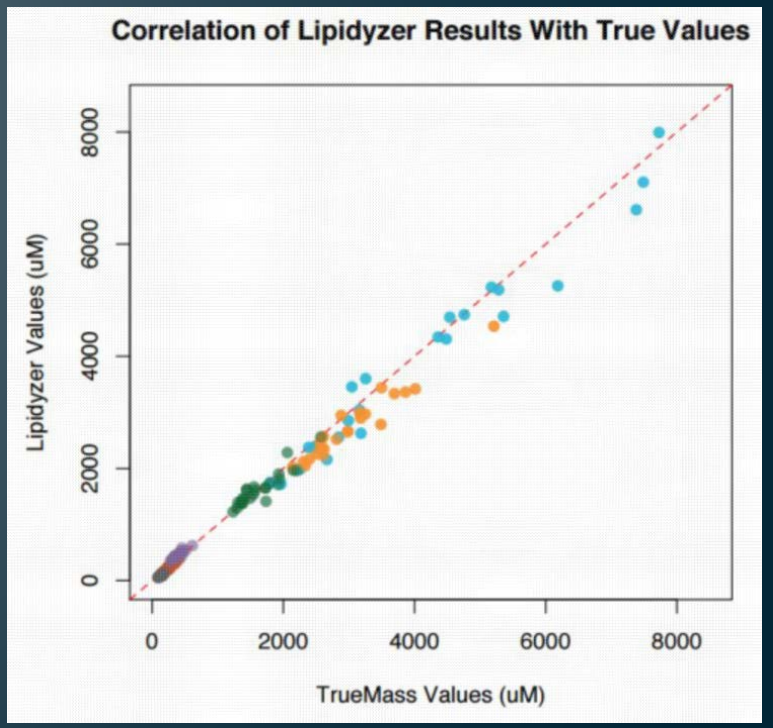
- ~140 known aqueous metabolites measured
- 25 major metabolite pathways measured
- 24 metabolites quantified absolutely [M]
- QC of CV ~ 5-8%



Serum Profiling of Lipids

- New targeted lipidomics platform measures up to 1200 lipids in 10 classes with absolute quantitation.
- CV: 5% accuracy: 10%

ceramides (CER)
cholesterol esters (CE)
diacylglycerols (DAG)
free fatty acids (FFA)
lysophosphatidylcholines (LPC)
lysophosphatidylethanolamines (LPE)
phosphatidylcholines (PC)
phosphatidylethanolamines (PE)
sphingomyelins (SM)
triacylglycerols (TAG)



Urine Profiling

GC-MS

- 50 – 100 identified species
- ~ 200 unidentified metabolite features
- CV ~ 20%

NMR

- 50 identified species
- Total spectral sum information
- CV ~ 5%

Potential Macronutrient Biomarkers

Total energy

- Doubly labeled water, weight variability
- Correlation with 'consumed' energy…**0.72**
- No improvement by including metabolomic profile data, or by including age and BMI

Protein

- Urinary nitrogen, DLW, weight variability
- Correlation with consumed protein…**0.66**
- No improvement by including metabolomic profile data

Potential Macronutrient Biomarkers *(continued)*

Carbohydrate

- DLW, weight variability and 11 metabolomic variables
- Correlation of **0.63** with consumed carbohydrate

Alcohol

- 9 metabolomic variables plus age and BMI
- Correlation of **0.51** with 'consumed' alcohol

Fat

- DLW, weight variability and metabolomic variables (incl. urine volume adjustment)
- Correlation of **0.44** with consumed fat
- Will try analyses stratified on BMI next, as well as a 'build up' approach for fat subtypes

Current Research Agenda

- Apply the same metabolomic platforms to blood and 24-hour urine from the 450 OS women in first phase of NPAAS. Develop calibration equations for each nutritional variable for which a biomarker can be identified, and examine the association between calibrated consumption estimates and outcomes in WHI cohorts
- Apply the same metabolomic platforms to case and control blood and spot urine from women in the three 'bone' clinics, for novel nutritional biomarker comparisons (and for overall metabolomic profile comparisons) bypassing any use of dietary self-report data