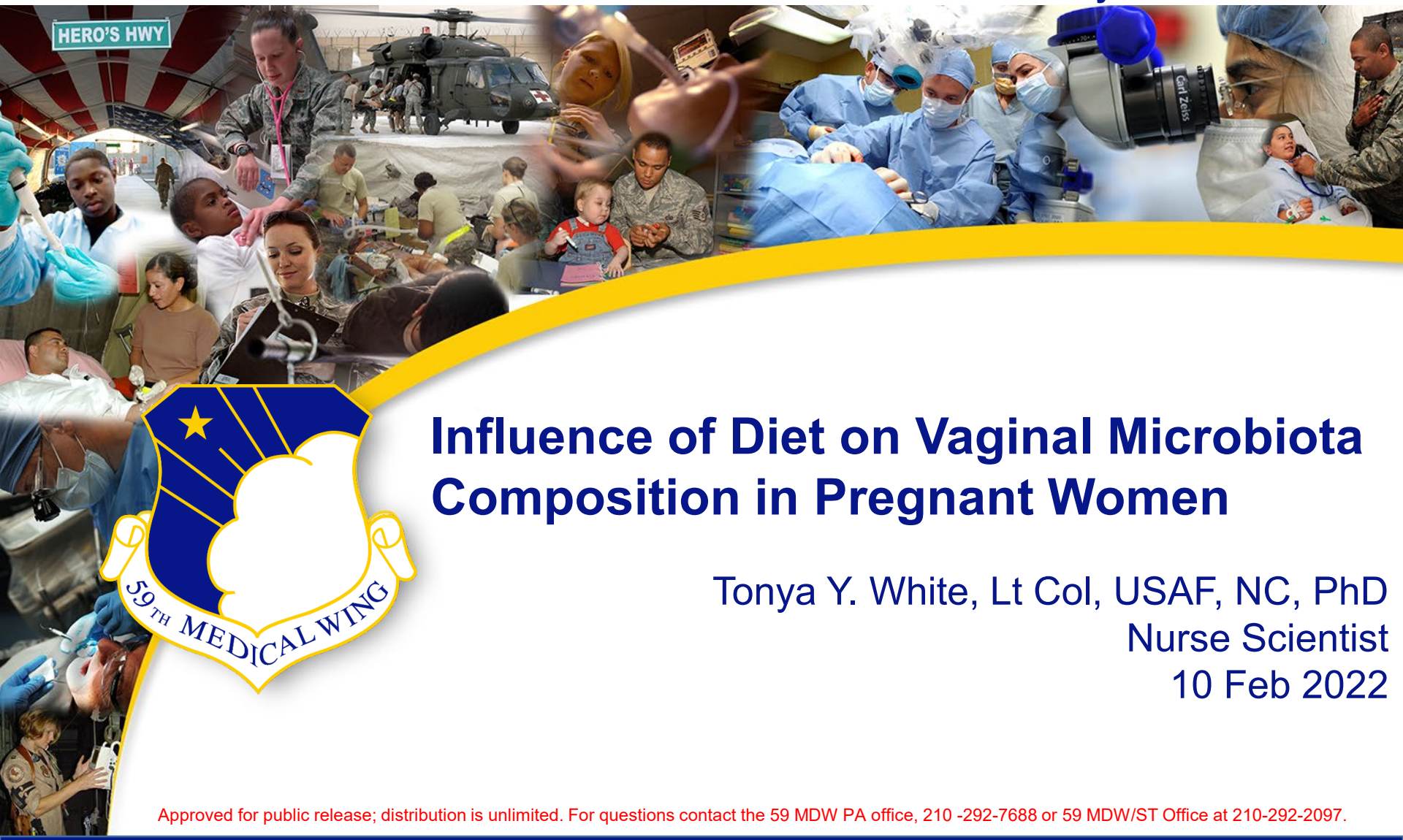
59th Medical Wing

Warrior Medics – Mission Ready – Patient Focused



Influence of Diet on Vaginal Microbiota Composition in Pregnant Women

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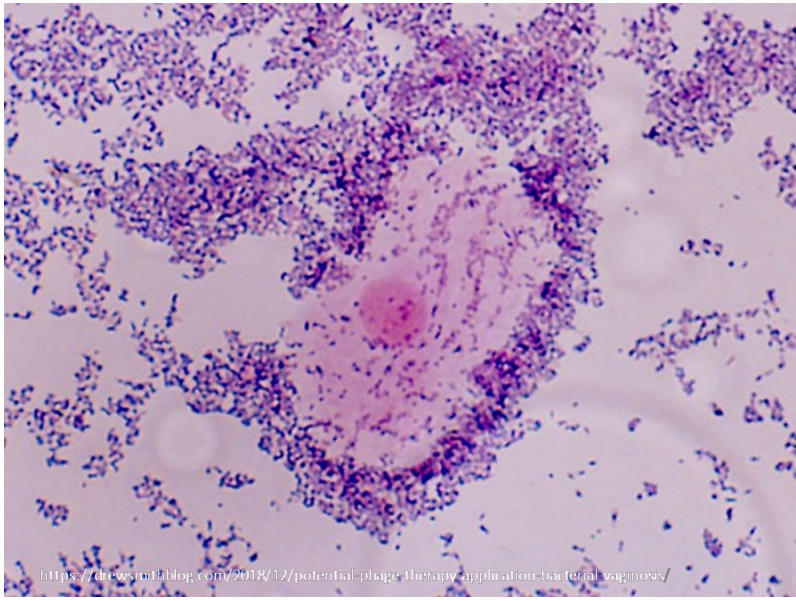
Disclosures

- The views expressed are those of the authors and do not reflect the official views or policy of the Air Force, the Department of Defense or its Components
- I acknowledge Dr. Mary Regan and Dr. Jacques Ravel for providing the data from their NINR funded study for this project/presentation
- I have no conflicts of interest to report

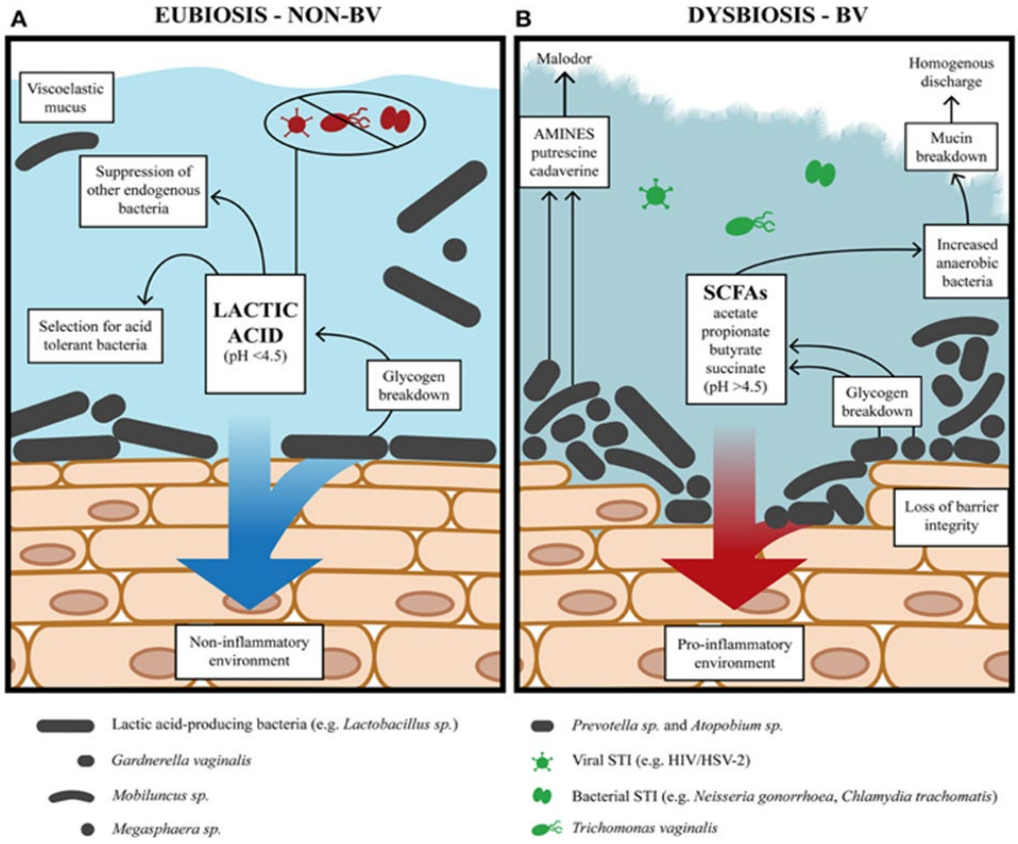
Introduction

- Bacterial vaginosis (BV) is a dysbiotic condition
- Affects 30% of reproductive age women
- Greater risk for Preterm birth and STIs
- As many as 80% are asymptomatic
- The economic burden is $15B
- Diet may play a role in shaping vaginal microbiota
- Understanding diet influences may have important clinical implications



BV Associated Dysbiosis

- Overgrowth of anaerobic and facultative bacteria
- Diverse polymicrobial composition
- Community Class type D



www.researchgate.net/figure/The-vaginal-environment-during-alternative-states-of-eubiosis-and-BV-A-During_fig1_278731618

Defining BV

- The etiology is not well understood
- Inconsistencies in the literature

Amsel-BV	BV meets at least 3 of 4 Amsel's criteria: • Abnormal discharge • pH>4.5 • Clue cells • Fish odort Symptomatic or Asymptomatic
Nugent-BV	BV diagnosed by Gram Stain: • Nugent score 7 – 10 (Nugent-BV) • Nugent score 4 - 6 (Intermediate-BV) Nugent score 0 – 3 (Non BV), *Lactobacillus*-dominated^ Symptomatic or Asymptomatic
Molecular-BV	General term for "non-optimal" bacterial communities depleted of lactobacilli with abundant anaerobes* characterized by molecular techniques
Seq BV	• 16S rRNA gene sequencing or broadrange PCR. Shotgun sequencing approaches High relative abundance of anaerobes* depleted of *Lactobacillus* spp. associated with increased genital inflammation and/or HIV risk*
qPCR-BV	• Taxon specific quantitative PCR "Non-optimal" taxa demonstrating concentration dependent associations with increased genital inflammation and/or odds of HIV risk Symptomatic or Asymptomatic
	^Depends on the population studied[22] *Polymicrobial/diverse or *G. vaginalis*-dominated #May also be associated with other adverse sexual as well as reproductive health outcomes

Mckinnon LR, Achilles SL, Bradshaw CS, Burgener A, Crucitti T, et al. 2019. The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. *AIDS Res. Hum. Retroviruses.* 35(3):219–28

Defining BV

- Amsel criteria
- Nugent score
- Sequencing

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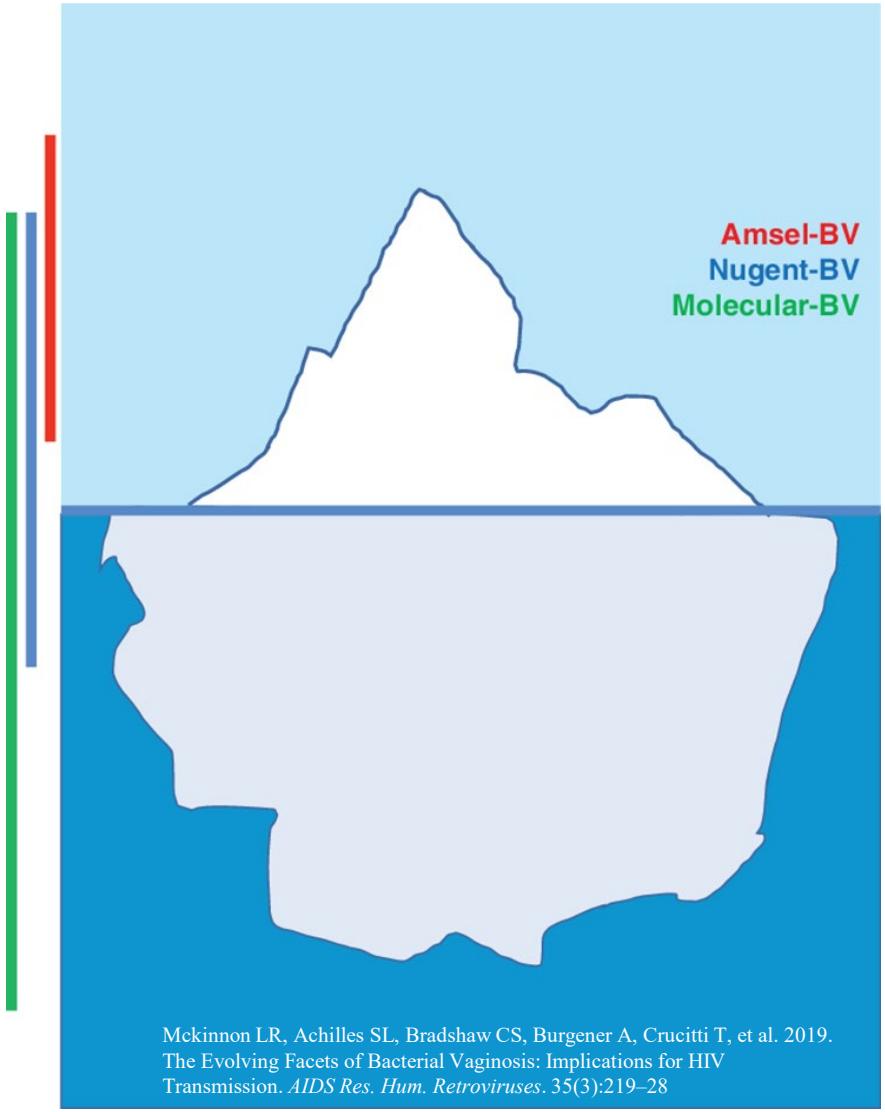
Molecular BV



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Literature Review

- Majority used Nugent scores to define BV
- Micronutrients associated with BV
- Macronutrients associated with BV
- Diet quality associated with BV
- Diet may shape the composition of the vaginal microbiota

Research Gaps

- Unclear what effect diet has on changes to the vaginal microbiota
- Inconsistencies in defining BV
- No studies have examined the longitudinal influence of diet on BV
- No studies on the influence of diet on molecular BV among pregnant women

Specific Aims

- Assess the influence of diet quality on the vaginal communities to determine if differences in diet quality were associated with molecular BV
- Analyze the relationship of diet quality scores and molecular BV using diet data at 6-, 7-, and 8-month gestations

Study Design

- Original analysis using longitudinal data collected prospectively in the *Birth, Eating, and the Microbiome* (BEAM, NR014826) Project
- The sample
- 18 to 34 years old
- Primigravid women with singleton pregnancies recruited between 12 to 22 weeks' gestation and were followed through delivery
- Inclusion criteria: participants who had complete dietary and vaginal microbiota data for gestational months 6, 7, and 8
- Exclusion criteria: more than one missing data point

Measures

Component HEI-2015 [1]	Max Points	Standard for Maximum Score	Standard for Minimum Score of Zero
Adequacy:			
Total Fruit [2]	5	≥ 0.8 cup equivalent/1000 kcal	No Fruit
Whole Fruit [3]	5	≥ 0.4 cup equivalent/1000 kcal	No Whole Fruit
Total Vegetables [4]	5	≥ 1.1 cup equivalent/1000 kcal	No Vegetables
Greens and Beans [4]	5	≥ 0.2 cup equivalent/1000 kcal	No Dark Green Vegetables or Beans or Peas
Whole Grains	10	≥ 1.5 ounce equivalent/1000 kcal	No Whole Grains
Dairy [5]	10	≥ 1.3 cup equivalent/1000 kcal	No Dairy
Total Protein Foods [6]	5	≥ 2.5 ounce equivalent/1000 kcal	No Protein Foods
Seafood and Plant Proteins [7,8]	5	≥ 0.8 ounce equivalent/1000 kcal	No Seafood or Plant Proteins
Fatty Acids [9]	10	(PUFAs + MUFAs)/SFAs > 2.5	(PUFAs + MUFAs)/SFAs ≤ 1.2
Moderation:			
Refined Grains	10	≤ 1.8-ounce equivalents/1000 kcal	≥ 4.3 ounce equivalent/1000 kcal
Sodium	10	≤ 1.1 g/1000 kcal	≥ 2.0 g per 1000 kcal
Added Sugar	10	≤ 6.5 % of energy	≥ 26 % of energy
Saturated Fats	10	≤ 8 % of energy	≥ 16 % of energy

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Vaginal Microbiota Measure

- VALENCIA
- Community Class Types
- Categorical variables
- L class for ‘non-BV group’
- D class for ‘molecular BV group’

Sample Characteristics

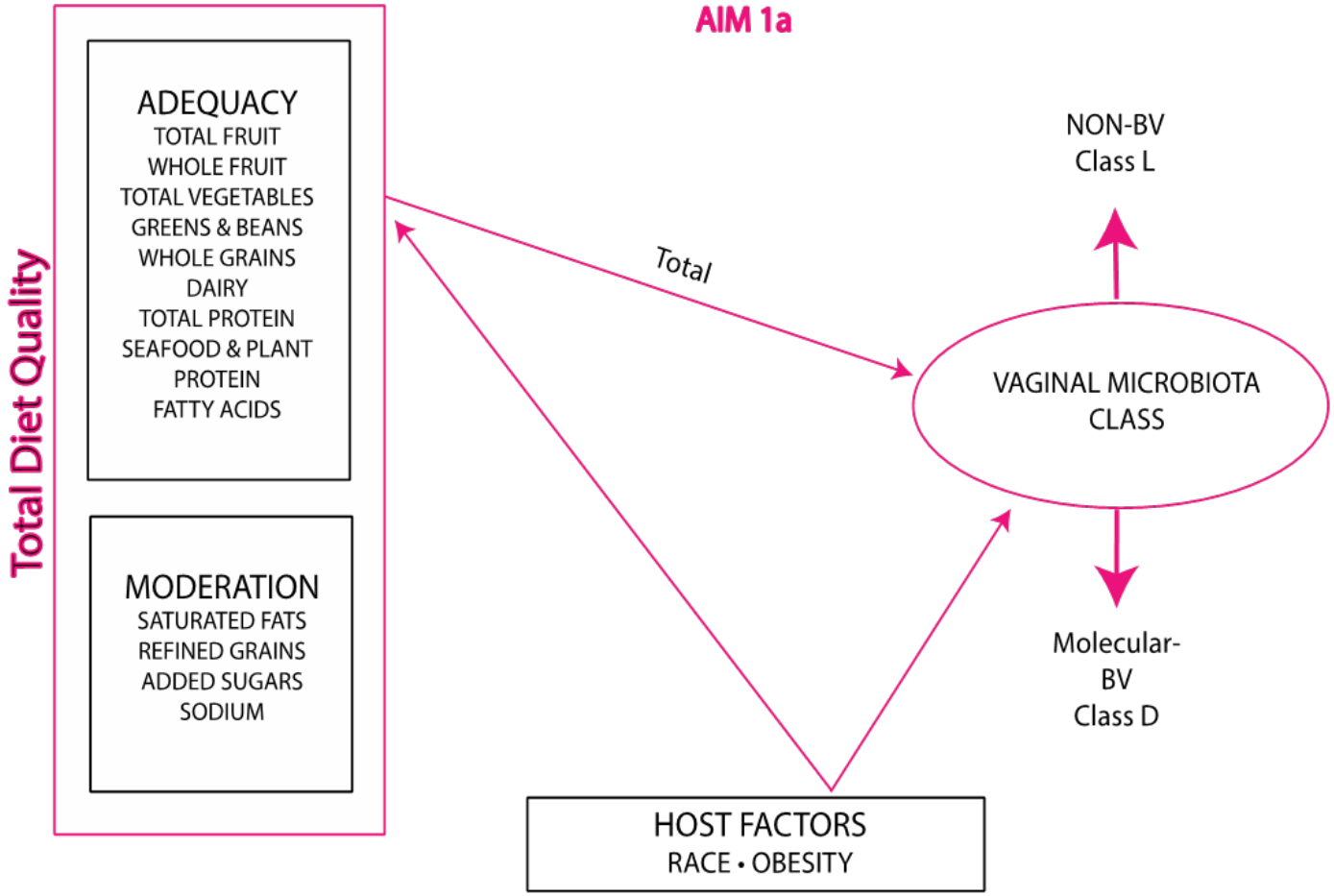
- Age range 18 -34
- Gestation at delivery 34 -41
- Body Mass Index 17.9 – 60, 40% BMI > 30
- Race, 71% African American
- Education, 36% university degree
- Household Income, 20% > $75K
- Employment status, 27% unemployed
- Marital status, 55% Single

https://www.nichd.nih.gov/ncmhep/initiatives/pregnancy-for-every-body/about

Specific Aim 1

- **Assess the influence of *total*, *adequacy*, and *moderation* diet quality scores on the vaginal communities among the cohort controlling for race and obesity**
  - Hypothesis – The higher diet quality scores would be associated with lower the odds of molecular BV and the lower the diet quality scores the higher the odds of molecular BV

Aim 1a


AIM 1a
Total Diet Quality
ADEQUACY
TOTAL FRUIT
WHOLE FRUIT
TOTAL VEGETABLES
GREENS & BEANS
WHOLE GRAINS
DAIRY
TOTAL PROTEIN
SEAFOOD & PLANT PROTEIN
FATTY ACIDS
MODERATION
SATURATED FATS
REFINED GRAINS
ADDED SUGARS
SODIUM
Total
NON-BV
Class L
VAGINAL MICROBIOTA CLASS
Molecular-BV
Class D
HOST FACTORS
RACE • OBESITY

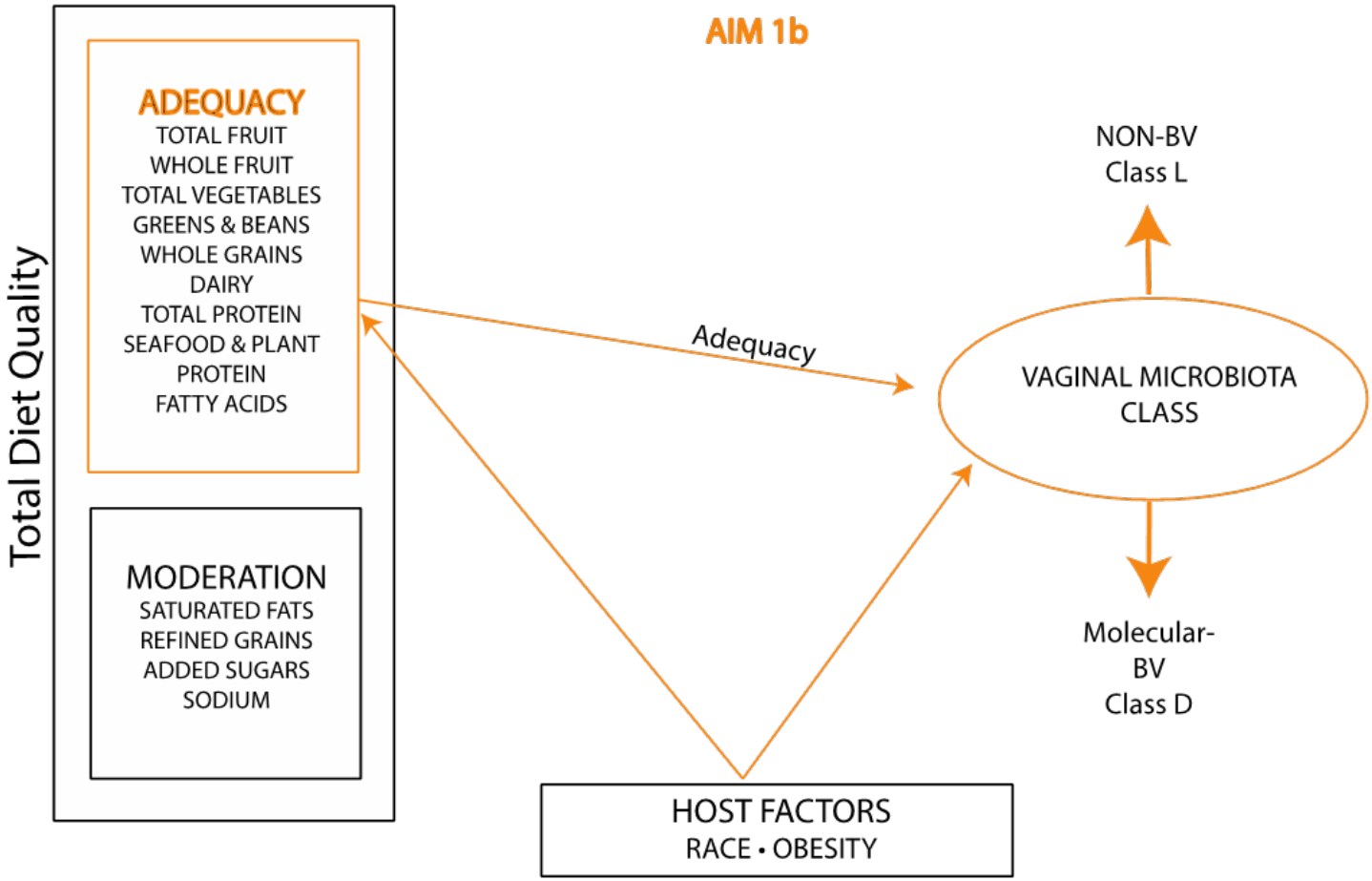
Aim 1a

n = 55	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Total diet score	-0.026	0.04	0.416	1	0.519	0.974	0.901	1.054
Obesity	-0.738	0.632	1.365	1	0.243	0.478	0.139	1.649
African Am.	1.127	0.822	1.881	1	0.17	3.086	0.617	15.444

Aim 1a

$n = 53$	β	SE	Wald	df	p	OR	95% CI	
							Lower	Upper
Total diet quality	-0.017	0.052	0.101	1	0.751	0.984	0.888	1.09
African American	-1.409	1.465	0.925	1	0.336	0.244	0.014	4.315
Single	0.883	0.901	0.96	1	0.327	2.419	0.413	14.148
Income > 75K	-2.449	1.434	2.919	1	0.088	0.086	0.005	1.434
University degree	0.73	1.161	0.396	1	0.529	2.076	0.213	20.222
Age	-0.182	0.093	3.782	1	0.052	0.834	0.695	1.001
Gestation	-0.659	0.285	5.358	1	0.021*	0.517	0.296	0.904
Forward Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Del	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958
Backward Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Del	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958

Aim 1b


AIM 1b
Total Diet Quality
ADEQUACY
TOTAL FRUIT
WHOLE FRUIT
TOTAL VEGETABLES
GREENS & BEANS
WHOLE GRAINS
DAIRY
TOTAL PROTEIN
SEAFOOD & PLANT PROTEIN
FATTY ACIDS
MODERATION
SATURATED FATS
REFINED GRAINS
ADDED SUGARS
SODIUM
Adequacy
NON-BV
Class L
VAGINAL MICROBIOTA CLASS
Molecular-BV
Class D
HOST FACTORS
RACE • OBESITY

Aim 1b

Table 10. Aim 1b, Model 1

n = 55	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Adequacy score	-0.023	0.049	0.211	1	0.646	0.978	0.887	1.077
Obesity	-0.679	0.62	1.197	1	0.274	0.507	0.15	1.711
African Am.	1.264	0.778	2.636	1	0.104	3.539	0.77	16.28

Aim 1b

n = 53	*β*	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Adequacy score	-0.02	0.065	0.097	1	0.756	0.98	0.863	1.113
African American	-1.358	1.411	0.927	1	0.336	0.257	0.016	4.082
Single	0.903	0.903	1.001	1	0.317	2.467	0.421	14.475
Income > $75K	-2.467	1.433	2.966	1	0.085	0.085	0.005	1.406
University degree	0.7	1.155	0.368	1	0.544	2.015	0.209	19.374
Age	-0.179	0.095	3.547	1	0.06	0.836	0.694	1.007
Gestation at Delivery	-0.656	0.286	5.284	1	0.022*	0.519	0.296	0.908
Forward Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Delivery	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958
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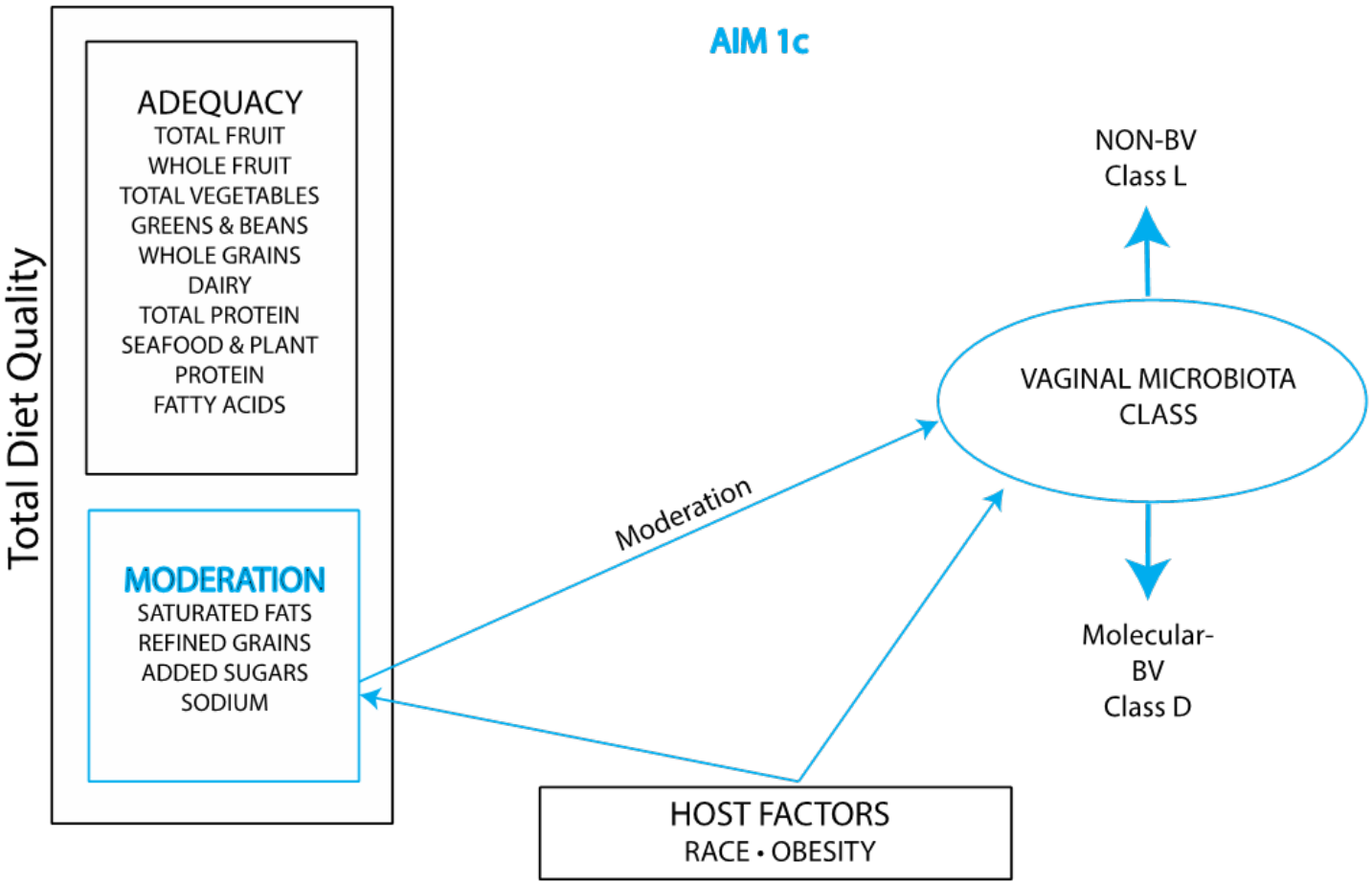
Aim 1c


AIM 1c
Total Diet Quality
ADEQUACY
TOTAL FRUIT
WHOLE FRUIT
TOTAL VEGETABLES
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Class D
HOST FACTORS
RACE • OBESITY

Aim 1c

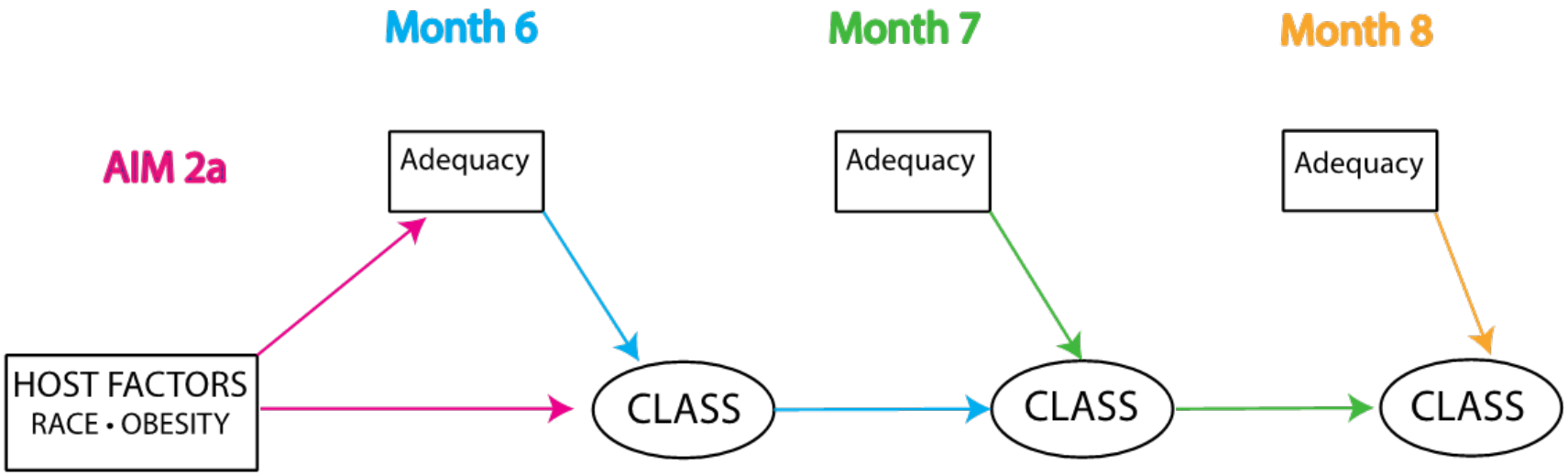
n = 55	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Moderation								
score	0.012	0.078	0.023	1	0.878	1.012	0.868	1.18
Obesity	-0.634	0.63	1.014	1	0.314	0.53	0.154	1.822
African Am.	1.472	0.808	3.32	1	0.068	4.356	0.895	21.208

Aim 1c

n = 53	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Moderation score	0.055	0.106	0.265	1	0.607	1.056	0.858	1.301
African Am.	-1.023	1.384	0.546	1	0.46	0.36	0.024	5.417
Single	0.89	0.9	0.978	1	0.323	2.435	0.417	14.203
Income > $75K	-2.622	1.486	3.114	1	0.078	0.073	0.004	1.337
University degree	0.592	1.157	0.262	1	0.609	1.808	0.187	17.453
Age	-0.185	0.091	4.13	1	0.042*	0.831	0.695	0.993
Gestation at Delivery	-0.649	0.29	4.992	1	0.025*	0.523	0.296	0.923
Forward Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Delivery	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958
Backwards Stepwise								
Age	-0.187	0.068	7.467	1	0.006*	0.83	0.726	0.949
Gestation at Delivery	-0.506	0.236	4.594	1	0.032*	0.603	0.379	0.958

Specific Aim 2

- **Analyze the relationship between a diet high in consumption of adequacy and moderation components and the community class using longitudinal diet and community class assignment data**
  - Hypothesis – The higher diet quality scores would be associated with lower odds of molecular BV and lower diet quality scores would be associated with higher the odds of molecular BV

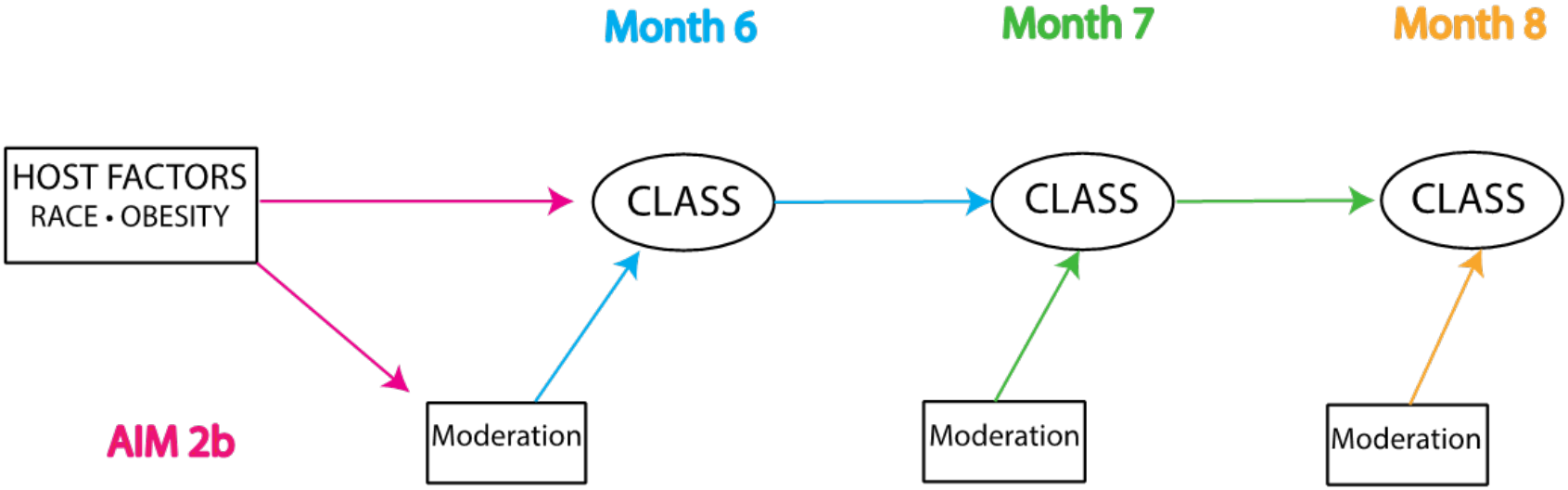

Month 6
Month 7
Month 8
AIM 2a
Adequacy
Adequacy
Adequacy
HOST FACTORS
RACE • OBESITY
CLASS
CLASS
CLASS

Aim 2a

n = 54	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Adequacy score	0.004	0.026	0.018	1	0.893	1.004	0.954	1.056
African American	0.929	0.6026	2.379	1	0.123	2.533	0.778	8.254
Obesity	-0.532	0.5259	1.024	1	0.312	0.587	0.209	1.646
6 Month	0.797	0.3462	5.294	1	0.021*	2.218	1.125	4.371
7 Month	0.056	0.249	0.05	1	0.822	1.058	0.649	1.723

n = 52	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Adequacy score	0.004	0.0288	0.019	1	0.889	1.004	0.949	1.062
Single	1.339	0.6979	3.681	1	0.055	3.816	0.972	14.983
Income > $75	-1.195	1.009	1.402	1	0.236	0.303	0.042	2.188
University Degree	2.062	0.8172	6.369	1	0.012*	7.864	1.585	39.012
Age	-0.109	0.0611	3.199	1	0.074	0.897	0.795	1.011
Gestation at Delivery	-0.598	0.173	11.971	1	0.001*	0.55	0.392	0.771
6 Month	1.035	0.4414	5.493	1	0.019*	2.814	1.185	6.684
7 Month	-0.06	0.3042	0.038	1	0.845	0.942	0.519	1.71

Specific Aim 2



Aim 2b

n = 55	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Moderation score	0.02	0.0324	0.39	1	0.532	1.02	0.958	1.087
African American	0.837	0.6405	1.706	1	0.191	2.308	0.658	8.1
Obesity	-0.498	0.5447	0.837	1	0.36	0.608	0.209	1.767
6 Month	0.947	0.3317	8.159	1	0.004*	2.579	1.346	4.94
7 Month	0.24	0.2617	0.843	1	0.359	1.272	0.761	2.124

Aim 2b

n = 53	ß	*SE*	Wald	*df*	*p*	OR	95% CI	
							Lower	Upper
Moderation score	0.03	0.038	0.603	1	0.437	1.03	0.956	1.11
Single	1.203	0.7744	2.413	1	0.12	3.33	0.73	15.19
Income > $75K	-1.615	1.0212	2.501	1	0.114	0.199	0.027	1.472
University Degree	2.248	0.8236	7.452	1	0.006*	9.471	1.885	47.57
Age	-0.125	0.0616	4.146	1	0.042*	0.882	0.782	0.995
Gestation at Delivery	-0.615	0.1757	12.229	1	0.000*	0.541	0.383	0.763
6 Month	1.22	0.4288	8.101	1	0.004*	3.389	1.462	7.852
7 Month	0.215	0.3608	0.354	1	0.552	1.239	0.611	2.514

Assumptions

- Diet influences the composition of the gut microbiota that, in turn, shapes the composition of vaginal microbiota either systemically or proximally by way of the perineum
- Diet quality is related to diet scores

Military Implications

- Majority of women report experiencing vaginal symptoms in austere environments
- Symptoms interfere with duty
- BV is a significant predictor for repeat chlamydia infections

Nursing Implications

- Treatment of BV needs a systemic approach
- Modifiable factors
- Younger women at greater risk
- Nursing education linking microbiome to health outcomes are lacking
- Demographic characteristics associated with molecular BV

Limitations

- Underpowered
- Assumptions may not be true
- Generalizability of findings
- Self selected photos
- Self-collected vaginal swabs
- Could not isolate for pre- and pro-biotic foods
- Inability to control for other confounding variables

Stregnths

- First known longitudinal study of the influence of diet on molecular BV
- Leveraged data from $5M NINR funded project
- Expands on findings from the parent project

Questions?