

An Update on Findings from Adventist Health Study-2

Gary E. Fraser

Vegetarian Dietary Patterns

Definitions

Lifetime Dynamics

Classification of Dietary Status

| | Number | % | |
|-------------------------|--------|------|--|
| Vegan | 9,062 | 8.5% | |
| Lacto-Ovo Vegetarian | 30,103 | 31% | |
| Pesco- Vegetarian | 9,793 | 10% | |
| Semi- Vegetarian | 4,801 | 5.5% | |
| Non- Vegetarian | 42,410 | 45% | |



Changes with aging—a life-time approach



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Update on Total Mortality



All-cause, cancer, and other mortality. Hazard ratios (HR) comparing mortality in the Adventist Health Study-2 (AHS-2) vs. the NLMS study for Total and Black populations.

Adjusted for gender, smoking, education, and regional of residence. Listed p-values are from chi-squared tests of the null

hypothesis that HR=1.

Footnote: *The comparisons between AHS-2 and NLMS here, depend on age. Two ages are selected to illustrate these differences.

| Food Group | Unprocessed (or minimally-processed) | Moderately- processed | Highly-processed (or ultra-processed) | |
|------------------------------------|--|---|---|----------|
| Fruits | fresh fruits [frozen fruits]a | canned fruits cooked fruits dried fruits fruit salad fruit juices | [fruit jams, preserves] [fruit pie fillings] | Le of |
| Vegetables | fresh/raw vegetables cooked vegetables [frozen vegetables] | canned vegetables vegetable juice tomato soup tomato pasta sauce | French fries, hash browns, fried potatoes catsup | Do |
| Grains & cooked cereals; breads | oatmeal brown rice, millet | white rice cream of wheat whole grain breads pasta | white breads other breads (bagels, biscuits, corn bread—write in) gluten steaks | |
| Meat analogues | | Nuteena (LL) Harvest Burger (MS) Spicy Black Bean Burger (MS) Vegan Burger (fat free) (NT) | all other listed meat analogues | |

Level of Processing of dietary items.

Does that relate to mortality?

| Food Group | Unprocessed (or minimally-processed) | Moderately-processed | Highly-processed (or ultra-processed) |
|----------------|---|----------------------|--|
| Dairy products | milk (whole) | yogurt (whole) | cheese (American |
| | milk (reduced) | yogurt (reduced) | processed and |
| | | cottage cheese | cheddar) |
| | | cheese (low-fat, | cream cheese/spreads |
| | | Mozarella, | other (whipping |
| | | Ricotta) | cream/sour |
| | | coconut milk | cream) |
| | | | evaporated/condense |
| | | | d milk |
| | | | soy/imitation cheese |
| | | | soy/rice drinks |
| Meats | beef or lamb (main | hamburger/ground | processed red meat |
| | dish) | beef | processed white meat |
| | chicken or turkey | pork | |
| | white fish | canned tuna | |
| | salmon | | |
| | other fish (write in) | | |



Level of Processed Food in Diets & Total Mortality

--Adjusted for % energy from animal foods & other covariates (Orlich et al—Submitted to journal)



Update on Cardiovascular Disease

Risk Factors for CVD



Pounds







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PRELIMINARY, not for citation





Associations between major risk factors and dietary patterns among black participants in AHS-2

| | (Vegetarian and Non-veg | d Vegan) versus getarian) |
|---------------------------------|----------------------------|------------------------------|
| A. Not adjusted for BMI | OR | 95% CI |
| Hypertension- | 0.56 | 0.36,0.87 |
| Diabetes | 0.48 | 0.24,0.98 |
| High TC- | 0.42 | 0.27,0.65 |
| High LDL-C- | 0.54 | 0.33,0.89 |
| Low HDL-C- | 0.69 | 0.45,1.07 |
| High TAG- | 1.05 | 0.56,2.00 |
| Obesity, females | 0.38 | 0.22,0.65 |
| Obesity, males | 0.52 | 0.24,1.13 |
| Obesity, both sexes | 0.43 | 0.28,0.67 |
| Abdominal adiposity, females | 0.55 | 0.33,0.92 |
| Abdominal adiposity, males | 0.48 | 0.22,1.04 |
| Abdominal adiposity, both sexes | 0.54 | 0.36,0.82 |

Adjusted for age, gender (as appropriate), education, physical activity, sub-study indicator and BMI as indicated. –Hypertension is systolic BP >140mmHg and/or diastolic BP > 90mmHg or taking medication for high BP. -High TC is >200mg/dl and high LDL-C is >130mg/dl or taking cholesterol-lowering medications in either case; low HDL-C is, 40mg/dl (males) or, 50mg/dl (females); high TAG is, 150mg/dl. Obesity is BMI >30.0 kg/m²; abdominal adiposity is WC >88 cm in females and >102 in males.

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Figure 2-2 Comparison of Adventist/non-Adventist Mortality Ratios for First Event Definite Fatal CHD by Age.* Adventist and Stanford Study Non-Hispanic White Subjects.



* Average across calendar years applying inverse standard error weights to year-specific standard event rate ratios

Multivariate-adjusted Hazard Ratio (HR) of CVD MORTALITY by quintile of the 'Meat' protein factor and by age categories in 81 337 participants of the Adventist Health Study-2.

HRs estimated at the mean age of each age category.



Tharrey M, et al Int J Epidemiol, 2018



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Multivariate-adjusted Hazard Ratio (HR) of CVD MORTALITY by quintile of the 'Nuts-Seeds' protein factor and by age categories in 81 337 participants of the Adventist Health Study-2.

HRs estimated at the mean age of each age category.



Tharrey M et al, Int J Epidemiol, 2018





Update on Selected Common Cancers



Incidence of all, common and medium frequency cancers. Hazard ratios (HR) comparing rates in the Adventist Health Study-2 (AHS-2) vs. the SEER-NLMS (National Longitudinal Mortality Study) for Total and Black populations.

Adjusted for gender, smoking, education, and region of residence (SEER vs. non-SEER areas). Listed p-values are from chi -squared tests of the null hypothesis that HR=1.

Specific Common Cancers and Vegetarian Dietary Patterns







Adjusted for: Race, Adult Height, Physical Activity, Screening, Educational Level, Smoking, Alcohol, Family History of Breast or Ovarian Cancer, Age at Menarche, Menopause Status, Age at Menopause, Number of Births, Breast Feeding, Use of Oral Contraceptives.

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Processed Meat, Dairy, and Colorectal Cancer

Selected Specific Foods





Adventist Health Study 2 Multivariate adjusted hazard ratio (HR) of the association between **Calcium intake & Milk** and incident **cancers of the colon and rectum** among participants in the Adventist Health Study-2^a

| | Extreme | Colorectal Cancer | | Colon Cancer | | |
|-----------------------------------|--------------------------|-------------------|-------------|--------------|-------------|--|
| | quintile ^a of | HR | 95%CI | HR | 95%CI | |
| | intake | | | | | |
| Dairy milk | Dairy milk 0 g/d | | | 1.00 | | |
| | 372 g/d | 0.63 | 0.43 - 0.89 | 0.67 | 0.45 - 0.99 | |
| | | | | | | |
| Total Calcium(BC) ^b | 468 mg/d | 1.00 | | 1.00 | | |
| custum(rro) | 1385 mg/d | 0.63 | 0.30 - 1.08 | 0.47 | 0.17 - 0.88 | |

^aExcept for regression calibration analyses quintiles are from food frequency questionnaire data.

^b Model 1: Total calcium, dairykcals, fiber, unprocessed red meat, processed red meat, fish, poultry, gender, race, BMI, education, alcohol consumption, cigarette smoking history, diabetes, use of aspirin, use of statin, physical activity, family history of colorectal cancer, history of polyps, screening for colorectal cancer.

Tantamango-Bartley Y, et al, Pub Health Nutr, 2017





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Dairy and Risk of Breast Cancer

SOY/DAIRY & BREAST CANCER

SOY AND BREAST CANCER

--*comparing extreme quintiles of intake

Adjusted for dairy kcals or dairy milk and much else.

| Soy Exposure | RR | (95% CI) | p for trend |
|----------------------|------|-------------|-------------|
| | | | |
| Total isoflavones* | 0.91 | (0.73-1.12) | 0.35 |
| Dietary isoflavones* | 0.95 | (0.77-1.18) | 0.64 |
| Supplemental | | | |
| isoflavones* | 0.63 | (0.35-1.14) | 0.13 |
| Soy milk* | 1.02 | (0.86-1.21) | 0.83 |

Not Statistically Significant







| Dairy Exposure | RR | (95% CI) | p for trend |
|---|-------------------|--------------------------|------------------------|
| Total subjects | | | |
| Total Dairy (kcals) | <mark>1.24</mark> | <mark>(1.02-1.51)</mark> | <mark>0.031</mark> |
| Dairymilk | <mark>1.37</mark> | <mark>(1.13-1.67)</mark> | <mark>0.001</mark> |
| Dairy Cheese | 0.94 | (0.74-1.20) | 0.61 |
| Yogurt | 1.01 | (0.85-1.21) | 0.88 |
| | | | |
| Substitution Analyses | | | |
| Dietary isoflavones/ dairy (substituting medians of | | | |
| users) | <mark>0.58</mark> | <mark>(0.40-0.85)</mark> | <mark>0.005</mark> |
| Soy milk/dairy milk (substituting medians of users) | <mark>0.65</mark> | <mark>(0.51-0.83)</mark> | <mark><0.001</mark> |

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DAIRY AND BREAST CANCER

(comparing extreme quintiles of intake)

--adjusted for soy, calcium and much else



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Dairy and Risk of Prostate Cancer

| Ta | Table 3:Relative hazard of incident prostate cancer, by tumor classification and race | | | | | | | |
|----|---|--|----------------------------|-------------------------------|--|--|--|--|
| | | Hazard Ratio (95% CI; p-value) | | | | | | |
| | | All prostate cancers Advanced cases Non-Advanced cases | | | | | | |
| | All participants (N=28 | ,737; all cases=1254, advanced | cases=190, non-advanced of | cases=1043; 21 uncategorized) | | | | |
| | Total calcium | 1.08 (0.92-1.27; 0.34) | 1.19 (0.78-1.81; 0.42) | 1.09 (0.91-1.30; 0.36) | | | | |
| | Supplemental calcium | 1.07 (0.91-1.26; 0.41) | 1.32 (0.86-2.00; 0.20) | 1.06 (0.89-1.26; 0.52) | | | | |
| | Dietary calcium | 1.14 (0.98-1.31; 0.08) | 1.03 (0.71-1.50; 0.87) | 1.17 (1.00-1.37; 0.05) | | | | |
| | Non-dairy calcium | 1.16 (0.94-1.44; 0.17) | 0.95 (0.54-1.67; 0.86) | 1.22 (0.97-1.55; 0.09) | | | | |
| | Dairy calcium | 1.60 (1.22-2.09; 0.0006) | 1.41 (0.72-2.76; 0.32) | 1.67 (1.25-2.24; 0.0007) | | | | |
| | Total dairy (g/day) | 1.65 (1.29-2.11; 6.89e-5) | 1.87 (0.99-3.50; 0.05) | 1.67 (1.27-2.19; 0.0002) | | | | |
| | Dairy energy (kcal/day) | 1.69 (1.30-2.21; 0.0001) | 1.76 (0.90-3.48; 0.10) | 1.74 (1.30-2.32; 0.0002) | | | | |

Black Subjects: Dairy Consumption and Prostate Cancer

| | Hazard Ratio (95% CI; p-value) | |
|-------------------------|--------------------------------|--|
| | Non-Advanced cases | |
| Black participants (N | N=6389; all cases=328, advance | d cases=41 ⁱ , non-advanced cases=283, 4 uncategorized) |
| Total calcium | 1.01 (0.72-1.42; 0.94) | 1.05 (0.73-1.52; 0.80) |
| Supplemental calcium | 1.05 (0.75-1.48; 0.77) | 1.05 (0.72-1.52; 0.80) |
| Dietary calcium | 1.13 (0.84-1.52; 0.41) | 1.23 (0.90-1.69; 0.20) |
| Non-dairy calcium | 1.02 (0.67-1.57: 0.92) | 1.06 (0.67-1.68: 0.81) |
| Dairy calcium | 1.59 (0.94-2.66: 0.08) | 1.91 (1.08-3.38: 0.03) |
| Total dairy (g/day) | 1.57 (0.96-2.55: 0.07) | 1.85 (1.09-3.15; 0.02) |
| Dairy energy (kcal/day) | 1.67 (0.98-2.83; 0.06) | 2.00 (1.12-3.58; 0.02) |



Diet, Molecules and Mechanisms: The "–omics"

---DNA methylation ---Metabolomics

How Does a Vegetarian Relative to a Non-vegetarian Diet Influence the....



Microbiome?



Metabolome



Methylome?



Table 2. Estimated non-null and observed differentially methylated genes (FDR < 0.05) summarized according to gene region or in relation to CpG islands comparing vegans with non-vegetarians In Vegans Detected: **Total Genes Estimated Non-null** Hyper-Hypo-% of all % of region-specific Region² total genes n n n n 18627 100 1081 5.8 4 14 53.7 388 3.9 **TSS200** 10008 4 4 13373 71.8 954 7.1 **TSS1500** 2 7 3' UTR 1935 10.4 55 2.8 1 2 **5' UTR** 41.3 475 6.2 7686 2 6 Gene Body 12072 64.8 1100 9.1 7 14 6262 33.6 405 6.5 9 1st Exon 1 8049 43.2 449 5.6 Intergenic 1 7 Island-related 73.5 649 4.7 **CpG** Island 13688 4 2 **North Shelf** 2562 13.8 180 7.0 2 2 **North Shore** 8315 44.6 424 5.1 1 7 South Shelf 2278 12.2 146 6.4 3 7 38.3 441 6.2 South Shore 7137 5 7 **Open Sea** 11884 63.8 798 6.7 2 14 459 5.0 Promoter 9131 49.0 2 9

• In vegans majority of differentially methylated genes were relatively hypomethylated, which is typically associated with increased gene expression.

• Perhaps explained in part by inhibition of DNA Methyltransferase enzymes by polyphenols

• More genes differentially methylated between vegans and non-vegetarians in the gene body (9%), but estimated at 4-6% in most gene regions.

- Differentially methylated genes that we detected have roles in:
- A. Protein synthesis
- RNA transport and regulation of translation
- Lysosome assembly
- Protein degradation or ubiquitination
- B cell receptor signaling
- A. Cellular Control (Relevant to cancer)
 - Tumor suppressor gene
 - DNA repair

How Does a Vegetarian Relative to a Non-vegetarian Diet Influence the....



Microbiome?



Metabolome



Methylome?



How Does a Vegetarian Relative to a Non-vegetarian Diet Influence the....



Microbiome?



Metabolome



Methylome?



Most Influential Differentially Abundant Metabolites Comparing Vegans and Non-vegetarians

| 3-bromo-5-chloro-2.6-dihydroxybenzoic acid* | | | | | | | | · · · · o · · · · |
|---|---------------|---|----|--|----|-------------|-----|-------------------|
| 3-methylhistidine | | | | | | | 0 | |
| 1-methyl-5-imidazoleacetate | | | | | | ·····o····· | | |
| n n-trimethyl-5-aminovalerate | | | | | | 0 | | |
| sphingomyelin (d18:1/17:0_d17:1/18:0_d19:1/16:0) | | | | | 0 | | | |
| 3.5 dichloro 3.6 dibudrovuhenzoid ecid | | | | | | | | |
| (1.4 or 15) methylaelmitete (e17:0 or i17:0) | | | | | | | | |
| 2 optional 4 motival 5 propul 2 furopproposite (ompt) | | | | ····· | - | | | |
| 5-carboxy-4-netriyi-5-propyi-2-turanpropanoate (cmpt) | | | | | | | | |
| neptenedioate (C7.1-dC)* | | | | ~ ~ ~ | | | | |
| margaroyicarnitine (c17)* | | | | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ | | | | |
| spningomyelin (d18:1725:0, d19:0724:1, d20:1723:0, d19:1724:0)* | | | | | | | | |
| undecenoyicarnitine (c11:1) | | | |) | | | | |
| sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)* | | | - | | | | | |
| perfluorooctanesulfonate (pfos) | | | | | | | | |
| (12 or 13)-methylmyristate (a15:0 or i15:0) | | | 0 | | | | | |
| 1-linoleoyl-2-linolenoyl-gpc (18:2/18:3)* | | C | | | | | | |
| 1-(1-enyl-stearoyl)-2-arachidonoyl-gpe (p-18:0/20:4)* | | ••••• | | | | | | |
| 1-methylurate | | 0 | | | | | | |
| paraxanthine | | •••••• | | | | | | |
| theophylline | | ••••• | | | | | | |
| ceramide (d18:1/17:0, d17:1/18:0)* | | ••••• | | | | | | |
| 1-lignoceroyl-gpc (24:0) | | ••••• | | | | | | |
| propionylcarnitine (c3) | | ••••• | | | | | | |
| 7-methylxanthine | | ····· 0 · · · · · · · · · · · · · · · · | | | | | | |
| 3-hydróxybutyroylalycine | | 0 | | | | | | |
| 5-acetylamino-6-amino-3-methyluracil | | 0 | | | | | | |
| hydroxy-cmpf* | | · · o · · · · · · · · · · · · · · · · · | | | | | | |
| hydroxyproline | ····· 0····· | | | | | | | |
| sphingomyelin (d18:1/14:0_d16:1/16:0)* | ····· 0······ | | | | | | | |
| 2-aminoadinate | 0 | | | | | | | |
| - and the application | | | | | | | | |
| | | I I | 1 | I | 1 | I | I I | |
| | | 30 | 35 | 40 | 45 | 50 | 55 | |

MeanDecreaseAccuracy

AHS-2 Metabolomics Pilot Results



- ~400 metabolites differentially abundant (untargeted panel of 930)
- 27 subclasses differentially abundant, specifically

Top increases in vegans:

- Vitamin A Metabolism
- Inositol
- Primary bile acid
- Ketone bodies

Top decreases in vegans:

- Xanthine metabolism
- Ceramides
- Histidine metabolism
- Branched fatty acid metabolism



Wrap-up

- So, what is the essence of all this?
- Adventists are doing better than others in terms of total mortality, risk of many cancers, heart disease, diabetes, HT.
- Among Adventists, it is the vegetarians who are doing best in all these respects, also with lower BMI—even though on average the non-vegetarian Adventists eat relatively little meat and are health-conscious.
- We are finding **biological signals** in the **DNA** and among a wide range of **metabolites**, showing that the cells of vegans are bathed in fluids that are different. Thus, this nutritional information has a **biological and mechanistic base** although much remains to be explored.

What is it in a vegetarian diet that makes a difference?

- Lack of red meat
- Lack of dairy
- Extra **nuts**
- Possibly the wide variety of **plant polyphenols**
- Fewer highly processed foods
- Probably much more to be discovered

Do vegans do better than other vegetarians?

- **Overall No** (i.e. for total mortality)
- They do have **less** CVD, cancer of breast and prostate
- Not so good for colorectal cancer (?calcium), and they **possibly** have more hemorrhagic stroke (non-AHS-2 data). Dementia is unclear.
- Of various vegetarian patterns, pesco-vegetarians actually have the lowest mortality—though unclear yet whether this is due to the fish

So, what is a sensible person to do? (IMO)

- It is clear that a **vegetarian diet** is to be preferred
- Restrict highly **processed foods**
- Be sure to get small regular quantities of **nuts**
- Minimize **dairy**, but get plenty of **calcium** (plant sources)
- Occasional **fish**, if desired
- Over the age of 60 years, supplement with **B12**.
- Ensure adequate (plant) **protein** for bones —usually not a problem.
- Healthy choices for us will also help the **planet**!

