

SUPPLEMENTARY MATERIALS

MANUSCRIPT TITLE: Associations of Fecal Microbiota with Ectopic Fat in African-Caribbean Men

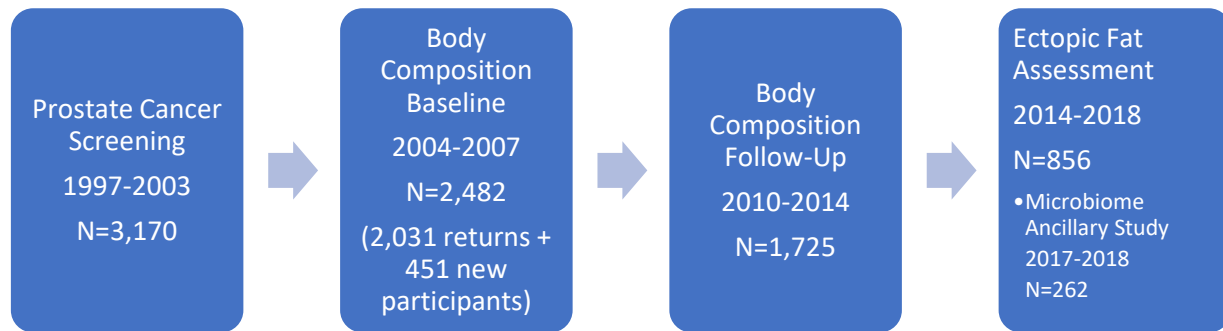
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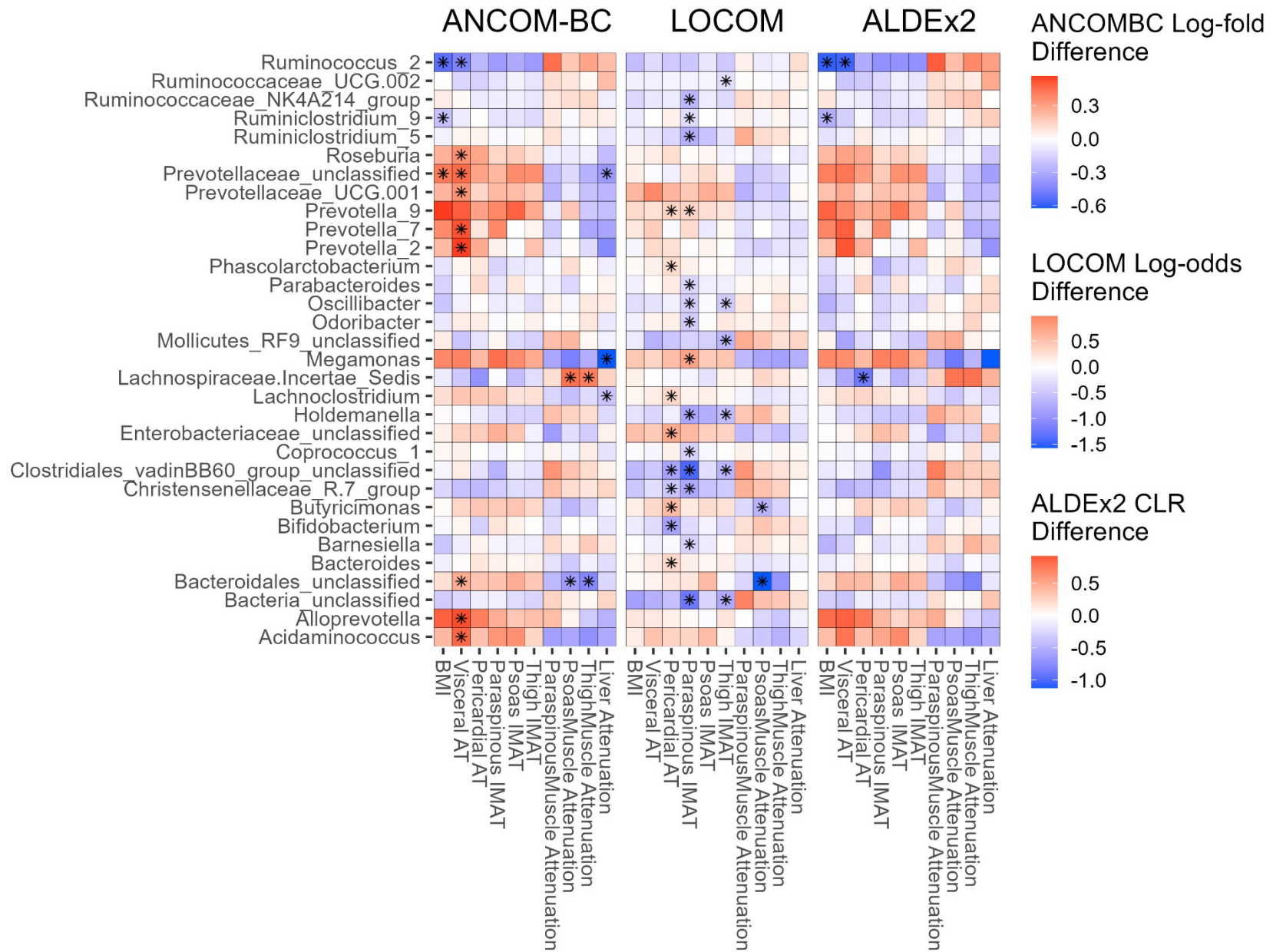
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Supplementary Figure S1 [title]: Study Flow Chart for the Tobago Health Study.

Supplementary Figure S1 [legend]: The first visit of the Tobago Health Study occurred between 1997-2003, which recruited men 40+ years who were ambulatory and not terminally ill. Between 2004-2007, men were invited to return for a (non-CT) body composition assessment visit; at this time, new participant recruitment also occurred using the same inclusion criteria as the first visit. Between 2010-2014, men were again invited to return for additional (non-CT) body composition measurements at a third study visit. A fourth study visit occurred between 2014-2018, in which a convenience sample of those attending the third study visit were invited for CT body composition measurements. Within this fourth study visit (between 2017-2018), we additionally implemented a fecal sample collection for microbiome assessment among a convenience sample of N=262 men who had already underwent CT body composition measurements.



Supplementary Figure S2 [title]: *Estimated changes in OTU abundance per standard deviation increment in BMI or fat, according to three differential abundance statistical approaches, among men from the Tobago Health Study.*

Supplementary Figure S2 [legend]: *A total of 193 men were included in the analyses. Rare (prevalence of <10%) and low-abundant (mean relative abundance <0.1%) microbes were pre-filtered from samples. Statistical models were analyzed separately for each BMI or fat measure; models adjusted for age (years), educational attainment (primary, secondary, or post-secondary), hours walked per week for exercise (hours), current smoking status (yes vs. no), drinking 4 or more alcoholic drinks per week (yes vs. no), and time difference between CT scans and fecal sample collection (years). Asterisks (*) denote statistically significantly associated OTUs, with different FDR-corrected q-value thresholds according to the model used (ANCOM-BC: $q < 0.05$; LOCOM and ALDEx2: $q < 0.20$). OTUs associated with at least one BMI or fat measure in at least one differential abundance testing model are included.*

Note: differential abundance models use different approaches (ex: linear regression, logistic regression) and different scaling of outcomes; thus, comparisons by strength of association across approaches is not appropriate. However, comparisons of directions of association, i.e., the heatmap colors, can be made.

(Left): Heatmap of ANCOM-BC results, showing the log-fold difference in microbial abundance per standard deviation increment in BMI or fat measure. (Middle): Heatmap of LOCOM results, showing the log-odds difference in microbial abundance per standard deviation increment in BMI or fat measure. (Right): Heatmap of ALDEx2 results, showing the CLR difference in microbial abundance per standard deviation increment in BMI or fat measure.

Abbreviations: OTU = operational taxonomic unit, ANCOM-BC = Analysis of Compositions of Microbiomes with Bias Correction, FDR = false discovery rate, IMAT = intermuscular fat, SD = standard deviation, LOCOM = logistic compositional analysis, CLR = center logratio

Supplementary Table S1: Sample characteristics comparing the N=262 men who returned for the Microbiome Visit compared to the remaining N=549 who did not, from the Tobago Cohort Study.

| Characteristic | Didn't Return for Microbiome Visit (N=594) | Returned for Microbiome Visit (N=262) | p-value |
|---|---|--|---------|
| Age (years) | 63.0 [57.0, 71.0] | 60.5 [56.3, 68.0] | 0.003 |
| African Caribbean Ethnicity | 545 (91.8%) | 236 (90.1%) | 0.999 |
| Missing | 6 (1.0%) | 8 (3.1%) | |
| Educational Attainment | | | |
| Primary | 423 (71.2%) | 179 (68.3%) | 0.337 |
| Post-Secondary | 80 (13.5%) | 29 (11.1%) | |
| Secondary | 81 (13.6%) | 44 (16.8%) | |
| Missing | 10 (1.7%) | 10 (3.8%) | |
| Hours Walked per Week | 1.5 [0.0, 4.5] | 2.0 [0.0, 5.6] | 0.546 |
| Missing | 7 (1.2%) | 3 (1.1%) | |
| Drinks 4+ alcohol/week (yes) | 81 (13.6%) | 31 (11.8%) | 0.511 |
| Current Smoking Status (yes) | 42 (7.1%) | 26 (9.9%) | 0.171 |
| Missing | 1 (0.2%) | 0 (0%) | |
| BMI (kg/m²) | 27.0 [24.3, 30.3] | 27.7 [25.0, 30.4] | 0.074 |
| Missing | 2 (0.3%) | 1 (0.4%) | |
| Alternative Mediterranean Diet Score (range 1-9) | 4.0 [3.0, 5.0] | 4.0 [3.0, 5.0] | 0.209 |
| Missing | 215 (36.2%) | 49 (18.7%) | |
| Energy intake (kcal/day) | 2497.1 [1839.6, 3195.3] | 2338.4 [1755.4, 3016.0] | 0.141 |
| Missing | 215 (36.2%) | 49 (18.7%) | |

Supplementary Table S5 [Footnote]: Sample descriptive statistics are provided for N=856 men from the Tobago Cohort Study who did (N=262) or did not (N=594) attend the ancillary microbiome study visit. Characteristics are presented as median (interquartile range) or N (%). P-values were calculated based on a Kruskal-Wallis or Fisher's exact test.

Supplementary Table S2: Age-adjusted partial Pearson correlations of BMI and fat measures, among men from the Tobago Health Study

| | VAT | Pericardial fat | Paraspinous IMAT | Psoas IMAT | Thigh IMAT | Paraspinous muscle attenuation | Psoas muscle attenuation | Thigh muscle attenuation | Liver Attenuation |
|--------------------------------|--------|-----------------|------------------|------------|------------|--------------------------------|--------------------------|--------------------------|-------------------|
| BMI | 0.67 † | 0.59 † | 0.49 † | 0.44 † | 0.75 † | -0.26 † | -0.21 † | -0.31 † | -0.51 † |
| VAT | | 0.69 † | 0.37 † | 0.49 † | 0.61 † | -0.19 † | -0.22 † | -0.31 † | -0.45 † |
| Pericardial fat | | | 0.39 † | 0.39 † | 0.57 † | -0.20 † | -0.32 † | -0.41 † | -0.32 † |
| Paraspinous IMAT | | | | 0.51 † | 0.57 † | -0.69 † | -0.46 † | -0.50 † | -0.19 † |
| Psoas IMAT | | | | | 0.45 † | -0.39 † | -0.50 † | -0.39 † | -0.23 † |
| Thigh IMAT | | | | | | -0.37 † | -0.35 † | -0.54 † | -0.33 † |
| Paraspinous muscle attenuation | | | | | | | 0.58 † | 0.65 † | 0.12 |
| Psoas muscle attenuation | | | | | | | | 0.72 † | 0.15 † |
| Thigh muscle attenuation | | | | | | | | | 0.14 † |

†: significant at $p < 0.05$

Supplementary Table S2 [Footnote]: A total of $N=193$ men were included in the analyses. Partial Pearson correlations with age adjustment were calculated. Statistically significant correlations at $p < 0.05$ is indicated with (†). Note: lower muscle or liver attenuation reflects greater lipid accumulation.

Abbreviations: VAT = visceral fat, IMAT = intermuscular fat

Supplementary Table S3: Age-by-BMI or age-by-fat interaction term p-values in alpha diversity models, among men from the Tobago Health Study

| BMI or Fat Measure | Observed OTUs | Pielou's Evenness | Shannon Diversity Index |
|--------------------------------|---------------|-------------------|-------------------------|
| BMI | 0.2759 | 0.7461 | 0.9264 |
| VAT | 0.0953 | 0.7697 | 0.2981 |
| Pericardial fat | 0.0844 | 0.1242 | 0.8736 |
| Paraspinous IMAT | 0.7303 | 0.5460 | 0.5146 |
| Psoas IMAT | 0.0287 | 0.9605 | 0.3816 |
| Thigh IMAT | 0.1230 | 0.9219 | 0.5107 |
| Paraspinous muscle attenuation | 0.8362 | 0.2403 | 0.6981 |
| Psoas muscle attenuation | 0.5668 | 0.0492 | 0.4218 |
| Thigh muscle attenuation | 0.0295 | 0.1434 | 0.8573 |
| Liver Attenuation | 0.7953 | 0.4329 | 0.3042 |

Supplementary Table S3 [Footnote]: A total of N=193 men were included in the analyses. P-values for interaction terms from models are presented. Statistically significant interactions at $p < 0.05$ are indicated with bold text. Multivariable models included age (years), educational attainment (primary, secondary, or post-secondary), hours walked per week for exercise (hours), current smoking status (yes vs. no), drinking 4 or more alcoholic drinks per week (yes vs. no), time difference between CT scans and fecal sample collection (years), unrarefied sequencing depth (in observed OTU models), and an interaction between age and alpha diversity metric.

Abbreviations: VAT = visceral fat, IMAT = intermuscular fat, OTU = operational taxonomic unit

Supplementary Table S4: Absolute difference in regression coefficients for fat measure predictors after adjustment for muscle volumes, among men from the Tobago Health Study

| BMI or Fat Measures | Observed OTUs | Pielou's Evenness | Shannon Diversity Index |
|--------------------------------|---------------|-------------------|-------------------------|
| Pericardial fat | 0.01 | 0.00 | 0.00 |
| Paraspinous IMAT | 0.00 | 0.00 | -0.01 |
| Psoas IMAT | 0.00 | -0.03 | -0.02 |
| Thigh IMAT | 0.01 | -0.03 | -0.02 |
| Paraspinous muscle attenuation | 0.01 | 0.00 | -0.01 |
| Psoas muscle attenuation | 0.01 | -0.02 | -0.01 |
| Thigh muscle attenuation | 0.00 | -0.01 | -0.01 |

Supplementary Table S4 [Footnote]: A total of $N=193$ men were included in the analyses. Fecal microbiota samples were first rarefied to a sequencing depth of 1,500 reads. Differences in regression coefficients (modeled as per SD increase in alpha diversity metric) were calculated as $\theta_{\text{not muscle volume adjusted}} - \theta_{\text{muscle volume adjusted}}$. Multivariable models included age (years), educational attainment (primary, secondary, or post-secondary), hours walked per week for exercise (hours), current smoking status (yes vs. no), drinking 4 or more alcoholic drinks per week (yes vs. no), time difference between CT scans and fecal sample collection (years), and unrarefied sequencing depth (in observed OTU models); in muscle volume adjusted models, the respective muscle volume (cardiac, paraspinous, psoas, thigh) was additionally adjusted.

Abbreviations: VAT = visceral fat, IMAT = intermuscular fat, OTU = operational taxonomic unit

Supplementary Table S5: Difference in adjusted R^2 from PERMANOVA models for fat measure predictors after adjustment for muscle volumes, among men from the Tobago Health Study

| Fat Measure | ΔR^2 |
|--------------------------------|--------------|
| Pericardial fat | 0.01% |
| Paraspinous IMAT | -0.01% |
| Psoas IMAT | 0.27% |
| Thigh IMAT | 0.30% |
| Paraspinous muscle attenuation | -0.03% |
| Psoas muscle attenuation | 0.13% |
| Thigh muscle attenuation | -0.02% |

Supplementary Table S5 [Footnote]: A total of $N=193$ men were included in the analyses. Fecal microbiota samples were first rarefied to a sequencing depth of 1,500 reads. Differences in adjusted R^2 were calculated as $R^2_{\text{not muscle volume adjusted}} - R^2_{\text{muscle volume adjusted}}$. Multivariable models included age (years), educational attainment (primary, secondary, or post-secondary), hours walked per week for exercise (hours), current smoking status (yes vs. no), drinking 4 or more alcoholic drinks per week (yes vs. no), and time difference between CT scans and fecal sample collection (years); in muscle volume adjusted models, the respective muscle volume (cardiac, paraspinous, psoas, thigh) was additionally adjusted. Abbreviations: PERMANOVA = permutational analysis of variance, IMAT = intermuscular fat, CT = computed tomography

Supplementary Table S6: Absolute difference in regression coefficients for BMI or fat measure predictors after adjustment for diet (N=164), among men from the Tobago Health Study

| BMI or Fat Measures | Not Fat and Diet Co-Adjusted | | | Fat and Diet Co-Adjusted | | | $\beta_{\text{not diet-adjusted}} - \beta_{\text{diet-adjusted}}$ | | |
|--------------------------------|------------------------------|-------------------|-------------------------|--------------------------|-------------------|-------------------------|---|-------------------|-------------------------|
| | Observed OTUs | Pielou's Evenness | Shannon Diversity Index | Observed OTUs | Pielou's Evenness | Shannon Diversity Index | Observed OTUs | Pielou's Evenness | Shannon Diversity Index |
| BMI | -0.02 | -0.15 | -0.13 | -0.02 | -0.15 | -0.13 | -0.01 | 0.00 | 0.00 |
| VAT | -0.13 | -0.20 | -0.19 | -0.13 | -0.21 | -0.20 | 0.00 | 0.01 | 0.01 |
| Pericardial fat | -0.04 | -0.13 | -0.12 | -0.05 | -0.14 | -0.13 | 0.01 | 0.01 | 0.01 |
| Paraspinous IMAT | -0.02 | -0.08 | -0.07 | -0.03 | -0.09 | -0.08 | 0.00 | 0.01 | 0.01 |
| Psoas IMAT | -0.01 | -0.05 | -0.05 | -0.01 | -0.06 | -0.05 | 0.00 | 0.00 | 0.00 |
| Thigh IMAT | -0.06 | -0.11 | -0.10 | -0.05 | -0.11 | -0.10 | -0.01 | 0.00 | 0.00 |
| Paraspinous muscle attenuation | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.00 | -0.01 | -0.01 |
| Psoas muscle attenuation | 0.03 | 0.01 | 0.01 | 0.04 | 0.01 | 0.02 | 0.00 | -0.01 | -0.01 |
| Thigh muscle attenuation | 0.03 | 0.04 | 0.04 | 0.04 | 0.06 | 0.05 | 0.00 | -0.02 | -0.01 |
| Liver Attenuation | 0.10 | 0.15 | 0.14 | 0.07 | 0.13 | 0.13 | 0.03 | 0.01 | 0.02 |

Supplementary Table S6 [Footnote]: A total of N=164 men with dietary intake data were included in the analyses. Differences in regression coefficients (modeled as per SD increase in alpha diversity metric) were calculated as $\beta_{\text{not diet-adjusted}} - \beta_{\text{diet-adjusted}}$. Multivariable models included age (years), educational attainment (primary, secondary, or post-secondary), hours walked per week for exercise (hours), current smoking status (yes vs. no), drinking 4 or more alcoholic drinks per week (yes vs. no), time difference between CT scans and fecal sample collection (years), and unrarefied sequencing depth (in observed OTU models); models with diet additionally adjusted for an alternative Mediterranean diet score and total energy intake (kcal/day).

†: alternative Mediterranean diet score and energy intake were both included in any diet-adjusted models. Change in β for diet measures is reported for BMI and VAT models, but was similar across all models.

Abbreviations: VAT = visceral fat, IMAT = intermuscular fat, OTU = operational taxonomic unit

Supplementary Table S7: Difference in % variance (adjusted R^2) explained by fat or diet predictors from PERMANOVA models, before and after co-adjustment of diet and fat measures (N=164), among men from the Tobago Health Study

| BMI, Fat, or Diet Measures | % Variance Explained, No Diet and Fat Co- Adjustment | % Variance Explained, With Fat and Diet Co- adjustment | ΔR^2 |
|--|--|--|--|
| BMI | 2.266% | 2.301% | -0.04% |
| VAT | 2.439% | 2.568% | -0.13% |
| Pericardial fat | 1.560% | 1.686% | -0.13% |
| Paraspinous IMAT | 0.900% | 1.016% | -0.12% |
| Psoas IMAT | 0.707% | 0.728% | -0.02% |
| Thigh IMAT | 0.832% | 0.844% | -0.01% |
| Paraspinous muscle attenuation | 0.358% | 0.380% | -0.02% |
| Psoas muscle attenuation | 0.247% | 0.233% | 0.01% |
| Thigh muscle attenuation | 0.568% | 0.707% | -0.14% |
| Liver Attenuation | 1.032% | 0.961% | 0.07% |
| Alternative Mediterranean Diet Score † | 0.543% | BMI Model: 0.522% VAT Model: 0.597% | BMI Model: -0.02% VAT Model: -0.05% |
| Energy Intake † | 0.615% | BMI Model: 0.649% VAT Model: 0.637% | BMI Model: -0.03% VAT Model: -0.02% |

Supplementary Table S7 [Footnote]: A total of N=164 men with dietary intake data were included in the analyses. Differences in adjusted R^2 were calculated as $R^2_{\text{not diet-adjusted}} - R^2_{\text{diet-adjusted}}$. Multivariable models included age (years), educational attainment (primary, secondary, or post-secondary), hours walked per week for exercise (hours), current smoking status (yes vs. no), drinking 4 or more alcoholic drinks per week (yes vs. no), and time difference between CT scans and fecal sample collection (years); models with diet additionally adjusted for an alternative Mediterranean diet score and total energy intake (kcal/day). †: alternative Mediterranean diet score and energy intake were both included in any diet-adjusted models. Change in % variance explained by diet is reported for BMI and VAT models, but was similar across all models.

Abbreviations: VAT = visceral fat, IMAT = intermuscular fat, OTU = operational taxonomic unit

Supplementary Table S8: β (95% CI) for the association of diet measures with a 1 standard deviation larger BMI, fat measures, and microbiome alpha diversity measures (N=164), among men from the Tobago Health Study

| Outcomes: BMI, Fat, or Alpha Diversity Measures | Predictor: Alternative Mediterranean Diet Score (per 1-unit increase) † | Predictor: Energy Intake (per 500 kcal) † |
|---|---|---|
| BMI | 0.01 (-0.08, 0.10) | -0.03 (-0.13, 0.07) |
| VAT | -0.02 (-0.12, 0.07) | -0.04 (-0.14, 0.06) |
| Pericardial fat | -0.05 (-0.14, 0.05) | -0.02 (-0.12, 0.09) |
| Paraspinous IMAT | -0.04 (-0.13, 0.05) | -0.04 (-0.14, 0.05) |
| Psoas IMAT | -0.01 (-0.10, 0.09) | -0.01 (-0.11, 0.09) |
| Thigh IMAT | 0.02 (-0.08, 0.12) | -0.05 (-0.15, 0.06) |
| Paraspinous muscle attenuation | 0.04 (-0.04, 0.12) | 0.07 (-0.02, 0.15) |
| Psoas muscle attenuation | 0.02 (-0.07, 0.11) | 0.04 (-0.06, 0.14) |
| Thigh muscle attenuation | 0.05 (-0.04, 0.14) | 0.06 (-0.03, 0.16) |
| Liver Attenuation | -0.10 (-0.19, -0.00) | 0.08 (-0.03, 0.18) |
| Observed OTUs | -0.09 (-0.19, 0.00) | 0.08 (-0.03, 0.18) |
| Pielou's Evenness | -0.06 (-0.16, 0.04) | -0.01 (-0.11, 0.10) |
| Shannon Diversity Index | -0.07 (-0.17, 0.02) | 0.01 (-0.09, 0.12) |

Supplementary Table S8 [Footnote]: A total of N=164 men with dietary intake data were included in the analyses. Multivariable linear regression models included adjustment for Alternative Mediterranean Diet score, energy intake (per 500 kcal/day), age (years), educational attainment (primary, secondary, or post-secondary), hours walked per week for exercise (hours), current smoking status (yes vs. no), drinking 4 or more alcoholic drinks per week (yes vs. no), time difference between CT scans and fecal sample collection (years), and unrarefied sequencing depth (in observed OTU model). Outcomes were standardized measures of BMI, fat, or alpha diversity. **Bold text** indicates a statistically significant association at $p < 0.05$