# **Supplementary material**

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**Supplementary Figure 1. Directed Acyclic Graph depicting the relationships between diet quality, the gut microbiome, and potential covariates.** The minimal adjustment set included age, smoking, medications which may impact the gut, intestinal symptoms, and physical activity.



**Supplementary Figure 2. Directed Acyclic Graph depicting the relationships between the gut microbiome, measures of muscle health, and potential covariates.** The minimal adjustment set included age, smoking, the Australian Recommended Food Score, The Dietary Inflammatory Index, medical conditions which may impact the gut, medical conditions which may impact muscle health, and physical activity.



**Supplementary Figure 3. Data-derived cut-offs for exploratory analyses.** Cut-offs for Skeletal muscle index tertiles were 7.8 kg/m2 and 9.0kg/m2 (A), the cut-off for high/low handgrip strength was 45kg (B), and the cut-off for high/low Timed Up-and-Go was 10 seconds (C).

**Supplementary Table 1. Associations of measures of alpha diversity with age, skeletal muscle index, timed up-and-go, and handgrip strength.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Age** | **B** | **95%CI** | **p value** |
| Shannon | -0.287 | -2.361, 1.788  | 0.786  |
| Observed  | 0.008  | -0.005, 0.021  | 0.226  |
| **Skeletal Muscle Index** |
| Shannon | -0.026 | -0.172, 0.119 | 0.724 |
| Observed  | -0.001 | -0.001, 0.000 | 0.251 |
| **Timed Up-and-Go** |  |  |  |
| Shannon | -0.133 | -0.543, 0.277 | 0.524 |
| Observed | 0.001 | -0.001, 0.004 | 0.377 |
| **Handgrip Strength** |
| Shannon | 0.803 | -0.287, 1.892 | 0.148 |
| Observed | 0.001 | -0.006, 0.008 | 0.784 |



**Supplementary Figure 4. Associations of alpha diversity with age, Skeletal Muscle Index, Timed Up-and-Go, and Handgrip Strength.** No associations were observed between either Observed or Shannon measures of alpha diversity and skeletal muscle index (A), handgrip strength (B), or timed up-and-go (C).



**Supplementary Figure 5. Principal coordinates analysis from unweighted and weighted UniFrac distances.** No significant differences were observed between participants in low and high categories for: (A) Skeletal muscle index (Unweighted UniFrac distance, PERMANOVA, p=0.213, Weighted UniFrac distance, PERMANOVA, p=0.448), (B) handgrip strength (Unweighted UniFrac distance, PERMANOVA, p=0.081, Weighted UniFrac distance, PERMANOVA, p=0.173), or (C) timed up-and-go (Unweighted UniFrac distance, PERMANOVA, p=0.253, Weighted UniFrac distance, PERMANOVA, p=0.266).



**Supplementary Figure 6.** Age stratified associations between butyrate-producing bacteria and muscle mass, strength, and function. No interaction with age was observed for handgrip strength or Timed Up-and-Go.

**Supplementary Table 2. Correlation matrix for muscle mass, strength, and function, and important contributing factors.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **SMI** | **TUG** | **HGS** | **ARFS** | **DII** | **Butyrate** | **Age** | **IRSAD** | **Education** | **Marital status** | **Employment**  |
| **SMI** | 1 | -0.232\*\* | 0.356\*\* | 0.116\* | -0.148\* | 0.187\*\* | -0.362\*\* | 0.171\*\* | 0.142\* | -0.041 | -0.291\*\* |
| **TUG** | -0.231\*\* | 1 | -0.443\*\* | -0.059 | 0.088 | -0.133\* | 0.525\*\* | -0.125\* | -0.273\*\* | 0.108\*\* | 0.366\*\* |
| **HGS** | 0.356\*\* | -0.443\*\* | 1 | 0.116\* | -0.179\*\* | 0.092\* | -0.583\*\* | 0.122\* | 0.227\*\* | -0.104\* | -0.433\*\* |
| **ARFS ^** | 0.116\* | -0.059 | 0.116\* | 1 | -0.457\*\* | 0.078 | -0.053 | 0.150\*\* | 0.177\*\* | -0.066 | -0.036 |
| **DII** | -0.148\* | 0.088 | -0.180\*\* | -0.457\*\* | 1 | -0.090\* | 0.118\* | -0.035 | -0.045 | 0.0370 | 0.109\* |
| **Butyrate** | 0.187\*\* | -0.133\* | 0.092\* | 0.078 | -0.090\* | 1 | -0.164\*\* | 0.123\* | 0.171\*\* | -0.078 | -0.170\*\* |
| **Age ^ #** | -0.362\*\* | 0.525\*\* | -0.583\*\* | -0.053 | 0.118\* | -0.164\*\* | 1 | -0.124\* | -0.240\*\* | 0.257\*\* | 0.694\*\* |
| **IRSAD** | 0.171\*\* | -0.125\* | 0.122\* | 0.150\*\* | -0.035 | 0.123\* | -0.124\* | 1 | 0.240\*\* | -0.042 | -0.099\* |
| **Education** | 0.142\* | -0.273\*\* | 0.227\*\* | 0.177\*\* | -0.045 | 0.171\*\* | -0.240\*\* | 0.240\*\* | 1 | -0.066 | -0.125\* |
| **Marital status** | -0.041 | 0.108\* | -0.104\* | -0.066 | 0.037 | -0.078 | 0.257\*\* | -0.042 | -0.066 | 1 | 0.113\* |
| **Employment**  | -0.291\*\* | 0.366\*\* | -0.433\*\* | -0.036 | 0.109\* | -0.170\*\* | 0.693\*\* | -0.099\* | -0.125\* | 0.113\* | 1 |
| **Smoking ^ #** | -0.117 | -0.066 | 0.053 | -0.064 | 0.032 | -0.050 | -0.121\*\* | -0.134\*\* | -0.063 | 0.034 | -0.056 |
| **PA ^ #** | 0.267\*\* | -0.414\*\* | 0.419\*\* | 0.204\*\* | -0.197\*\* | 0.248\*\* | -0.538\*\* | 0.122\* | 0.179\*\* | -0.113\* | -0.618\*\* |
| **Fat mass ^** | 0.289\*\* | 0.174\*\* | -0.114\* | -0.145\* | 0.109\* | 0.107\* | 0.101\* | 0.022 | -0.069 | -0.001 | 0.077 |
| **Intestinal symptoms #** | -0.063 | 0.042 | -0.013 | -0.041 | -0.087\* | -0.137\* | -0.087 | -0.095\*\* | -0.071 | -0.084 | -0.054 |
| **Stomachsymptoms** | -0.086 | 0.099\* | -0.026 | 0.001 | -0.001 | -0.136\* | 0.066 | -0.010 | -0.021 | -0.065 | 0.058 |
| **Muscle medical condition ^** | -0.084 | 0.208\*\* | -0.272\*\* | 0.003 | 0.068 | -0.065 | 0.350\*\* | -0.103\* | -0.116\* | 0.083 | 0.281\*\* |

**Supplementary Table 2 continued**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **SMI** | **TUG** | **HGS** | **ARFS** | **DII** | **Butyrate** | **Age** | **IRSAD** | **Education** | **Marital status** | **Employment**  |
| **Gut medical condition ^** | -0.162\*\* | 0.159\*\* | -0.142\* | -0.097\* | 0.020 | -0.115\* | 0.211\*\* | -0.069 | -0.090\* | 0.020 | 0.168\*\* |
| **Muscle medications** | -0.076 | 0.128\* | -0.157\*\* | -0.008 | 0.120\* | -0.138\* | 0.157\*\* | -0.140\* | -0.087\* | 0.080\* | 0.118\* |
| **Gut** **medications #** | -0.155\*\* | 0.257\*\* | -0.235\*\* | -0.120\* | 0.104\* | -0.157\*\* | 0.305\*\* | -0.116\* | -0.168\*\* | 0.008 | 0.257\*\* |
| **Batch ^ #** | 0.031 | 0.004 | 0.021 | 0.021 | -0.035 | 0.090\* | -0.074 | -0.067 | 0.004 | -0.045 | -0.068 |

**Supplementary Table 2 continued**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Smoking** | **PA** | **Fat mass** | **Intestinal symptoms** | **Stomach symptoms** | **Muscle med condition** | **Gut med condition** | **Muscle meds** | **Gut meds** | **Batch**  |
| **SMI** | -0.117\*\* | 0.267\*\* | 0.289\*\* | -0.063 | -0.086 | -0.084 | -0.162\*\* | -0.076 | -0.155\*\* | 0.031 |
| **TUG** | -0.066 | -0.414\*\* | 0.174\*\* | 0.042 | 0.099\* | 0.208\*\* | 0.159\*\* | 0.128\* | 0.257\*\* | 0.004 |
| **HGS** | 0.053 | 0.420\*\* | -0.114\* | -0.013 | -0.026 | -0.272\*\* | -0.142\* | -0.157\*\* | -0.235\*\* | 0.021 |
| **ARFS ^** | -0.064 | 0.204\*\* | -0.145\* | -0.041 | 0.001 | 0.003 | -0.099\* | -0.008 | -0.120\* | 0.021 |
| **DII** | 0.033 | -0.197\*\* | 0.109\* | -0.087 | -0.001 | 0.068 | 0.020 | 0.120\* | 0.104\* | -0.035 |
| **Butyrate** | -0.050 | 0.248\*\* | 0.107\* | -0.137\* | -0.136\* | -0.065 | -0.115\* | -0.138\* | -0.157\*\* | 0.090\* |
| **Age ^ #** | -0.121\* | -0.538\*\* | 0.100\* | -0.087 | 0.066 | 0.3500\*\* | 0.212\*\* | 0.157\*\* | 0.305\*\* | -0.074 |
| **IRSAD** | -0.134\* | 0.122\* | 0.022 | -0.095\* | -0.010 | -0.103\* | -0.069 | -0.140\* | -0.116\* | -0.067 |
| **Education** | -0.063 | 0.179\*\* | -0.069 | -0.071 | -0.021 | -0.116\* | -0.090\* | -0.090 | -0.168\*\* | 0.004 |
| **Marital status** | 0.034 | -0.113\* | -0.001 | -0.084 | -0.065 | 0.083 | 0.020 | 0.080 | 0.009 | -0.045 |
| **Employment**  | -0.056 | -0.618\*\* | 0.077 | -0.054 | 0.058 | 0.281\*\* | 0.168\*\* | 0.118\* | 0.257\*\* | -0.068 |
| **Smoking ^ #** | 1 | 0.021 | -0.124\* | 0.052 | 0.024 | -0.035 | -0.019 | -0.037 | -0.013 | 0.015 |
| **PA ^ #** | 0.021 | 1 | -0.209\*\* | -0.047 | -0.109\* | -0.219\*\* | -0.192\*\* | -0.120\* | -0.293\*\* | 0.052 |
| **Fat mass ^** | -0.124\* | -0.209\*\* | 1 | 0.004 | 0.039 | 0.144\* | 0.114\*\* | 0.061 | 0.175\*\* | 0.015 |
| **Intestinal symptoms #** | 0.052 | -0.047 | 0.004 | 1 | 0.349\*\* | 0.108\* | 0.182\*\* | 0.150\*\* | 0.134\* | -0.033 |
| **Supplementary Table 2 continued** |
|  | **Smoking** | **PA** | **Fat mass** | **Intestinal symptoms** | **Stomach symptoms** | **Muscle med condition** | **Gut med condition** | **Muscle meds** | **Gut meds** | **Batch**  |
| **Stomachsymptoms** | 0.024 | -0.109\* | 0.039 | 0.349\*\* | 1 | 0.124\* | 0.313\*\* | 0.145\* | 0.159\*\* | -0.037 |
| **Muscle medical condition ^** | -0.035 | -0.219\*\* | 0.144\* | 0.108\* | 0.124\* | 1 | 0.217\*\* | 0.337\*\* | 0.304\*\* | -0.016 |
| **Gut medical condition ^** | -0.019 | -0.192\*\* | 0.114\* | 0.182\*\* | 0.313\*\* | 0.217\*\* | 1 | 0.083 | 0.395\*\* | -0.021 |
| **Muscle medications** | -0.037 | -0.120\* | 0.061 | 0.150\*\* | 0.145\* | 0.337\*\* | 0.083 | 1 | 0.293\*\* | 0.052 |
| **Gut** **medications #** | -0.013 | -0.293\*\* | 0.175\*\* | 0.134\* | 0.159\*\* | 0.304\*\* | 0.395\*\* | 0.293\*\* | 1 | 0.086 |
| **Batch ^#** | 0.015 | 0.052 | 0.015 | -0.033 | -0.037 | -0.016 | -0.021 | 0.052 | 0.086 | 1 |
| ^Variables included in the minimum adjusted model for butyrate-producing bacteria and muscle health regression analysis, #Variables included in the minimum adjusted model for diet quality and butyrate-producing bacteria regression analysis. Correlations were conducted with Pearson correlation for continuous variables, Spearman correlation for categorical variables, and point-biserial for a combination of categorical, continuous, or binary variables. \*p<0.05, \*\*p<0.001. |