

SUPPLEMENTARY INFORMATION

Exercise and associated dietary extremes impact on gut microbial diversity

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Supplementary Table 1. Control physical activity assessment

| | BMI<25 | BMI >28 |
|--|--------|---------|
| Walking | | |
| winter (hours per week) | 6.26 | 4.30 |
| summer (hours per week) | 7.04 | 5.07 |
| Cycling | | |
| winter (hours per week) | 3.00 | 0.39 |
| summer (hours per week) | 3.61 | 0.98 |
| Gardening | | |
| winter (hours per week) | 0.28 | 0.21 |
| summer (hours per week) | 0.98 | 0.53 |
| Physical exercise e.g. aerobics, dancing swimming etc | | |
| winter (hours per week) | 1.16 | 0.61 |
| summer (hours per week) | 3.74 | 2.61 |
| DIY (hours per week) | 4.22 | 3.41 |
| Housework (hours per week) | 4.41 | 4.13 |
| How many hours weekly of vigorous activity (sweating/ ↑ heart rate) | 7.21 | 3.85 |
| No. flights of stairs per day | 7.17 | 9.09 |

Values shown are expressed as mean. t-test comparisons between low BMI and high BMI controls was undertaken.

Supplementary Table 2. Metabolic, inflammatory and other markers

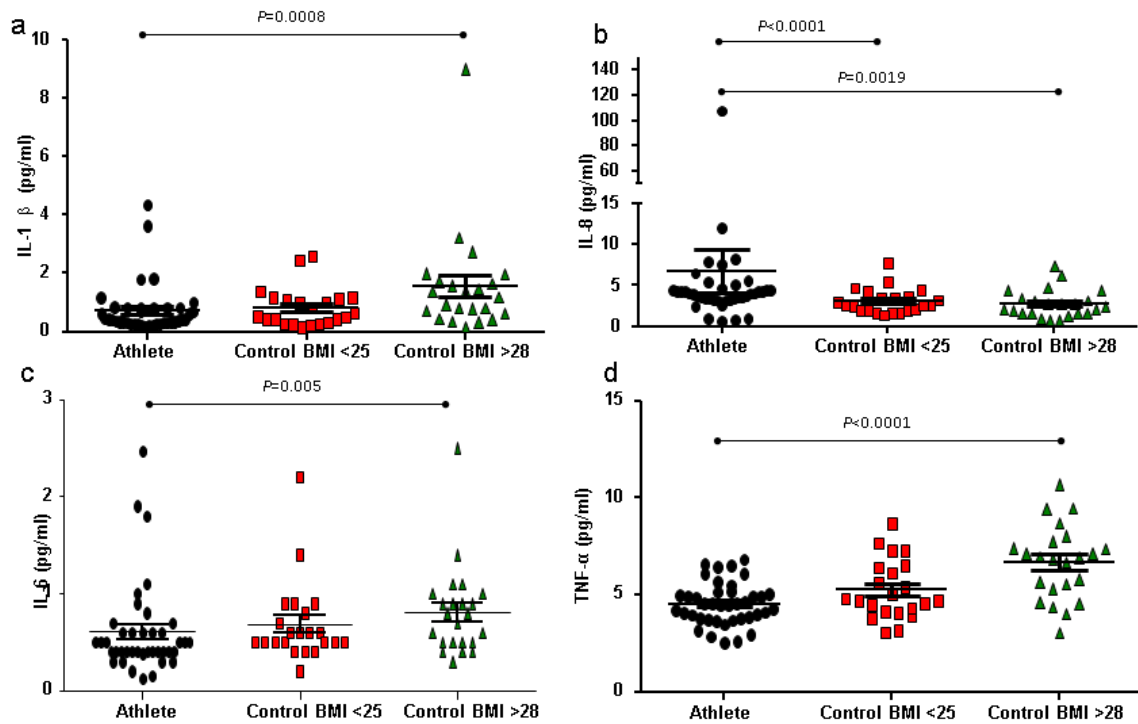
| | Athletes n=40 | Control BMI >28 n=23* | Controls BMI <25 n=23* |
|-----------------------------|--------------------------|---|--|
| Glucose (mmol/L) | 4.94±0.1 | 4.95±0.1* | 4.69±0.1* |
| CRP (mg/dL) | 1.12±0.1 | 1.94±0.5 | 2.18±0.7 |
| Adiponectin (ug/ml) | 7.02±0.39 | 8.5±0.6 | 9.8±0.6# |
| Insulin (pg/ml) | 227.88±24.6▲ | 469.1±84.9 | 490.3±123.04# |
| Leptin (pg/ml) | 1411.01±234.5▲ | 10382.5±2117.9† | 4237.1±1937.6 |
| IFN-γ (pg/ml) | 5.2±2.6 | 1.88±0.2 | 1.7±0.2 |
| IL-10 (pg/ml) | 8.78±3.8 | 2.67±0.3 | 63.5±53.4 |
| IL-12p70 (pg/ml) | 7.2±3.1 | 1.97±0.2 | 11.47±5.9 |
| IL-1B (pg/ml) | 0.5±0.05▲ | 1.56±0.4 | 5.6±4.8 |
| IL-6 (pg/ml) | 0.79±0.19▲ | 0.82±0.1 | 4.2±3.5 |
| IL-8 (pg/ml) | 10.45±5.8▲ | 2.79±0.4 | 3.2±0.3# |
| TNF-α (pg/ml) | 4.86±0.2▲ | 6.68±0.4 | 32.7±27.6 |
| Sodium (mmol/L) | 139±0.2▲ | 139.2±0.7 | 139.52±0.6 |
| Potassium (mmol/L) | 5.89±0.1▲ | 4.2±0.1† | 4.62±0.3# |
| Urea (mmol/L) | 8.57±0.2▲ | 5.2±0.2† | 6.07±0.2# |
| Creatine (μmol/L) | 97.42±2▲ | 82.44±2 | 88.48±3.9# |
| CK (IU/L) | 1038.17±112▲ | 159.44±26.9† | 389.52±105.7# |
| AST (IU/L) | 45.65±2.4▲ | 23.95±3.2 | 26.57±2.3# |
| YGT (IU/L) | 27.90±3.2 | 32.0±5.2† | 23.44±4.8# |
| Alkaline Phosphatase (IU/L) | 68.15±2.2 | 64.7±2.7 | 64.22±4.2 |
| Total Bilirubin (μmol/L) | 15.97±0.8 | 14.44±1.2 | 16.74±2.57 |
| Total Cholesterol (mmol/L) | 4.91±0.2 | 7.11±2.3 | 4.5±0.2 |
| HDL (mmol/L) | 1.44±0.04▲ | 1.2±0.4† | 1.39±0.05 |
| LDL (mmol/L) | 3.07±0.1 | 2.94±0.1 | 2.73±0.2 |
| Triglycerides (mmol/L) | 0.86±0.05▲ | 7.6±5.9† | 0.84±0.1 |
| Total Protein (g/L) | 71.03±0.5▲ | 73.95±0.9 | 73.74±0.8# |
| Albumin (g/L) | 41.45±0.3▲ | 43.8±0.6 | 44.44±0.4# |
| Globulin (g/L) | 29.58±0.43 | 30.2±0.7 | 29.3±0.7 |
| Calcium (mmol/L) | 2.43±0.01 | 2.41±0.2 | 2.34±0.1 |

Data is expressed as mean value ± standard error of mean. All intergroup comparisons were made using the Mann Whitney U test. *n=22, # p<0.05 between athletes and low BMI controls. ▲ p<0.05 between athletes and high BMI controls. † p<0.05 between high BMI and low BMI controls.

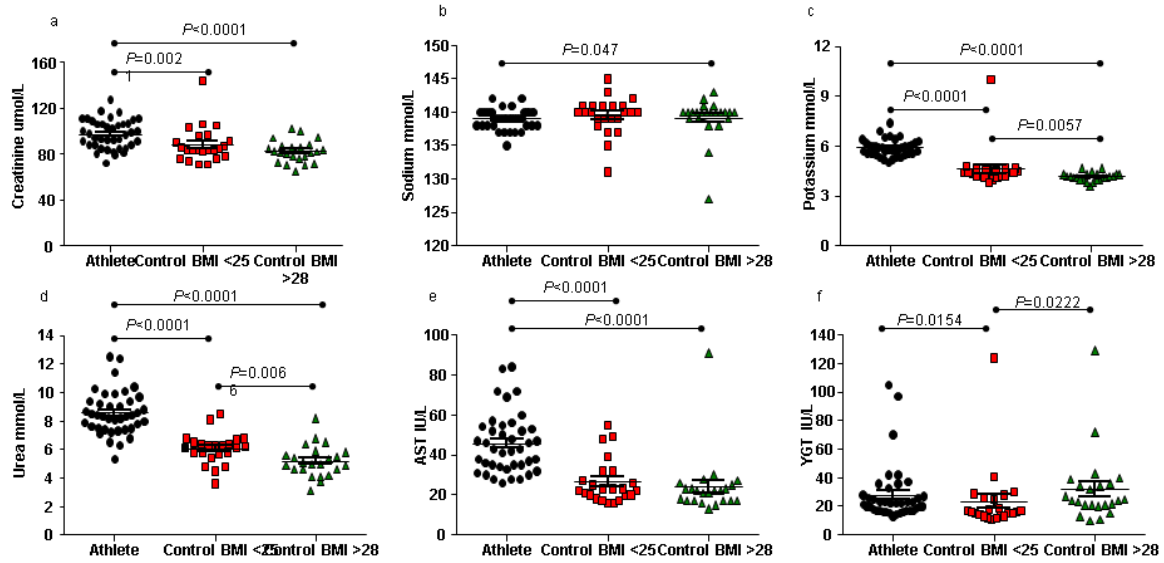
Supplementary Table 3 Microbiota composition in athletes and controls at phylum level

| | Low BMI Controls (n=23) | High BMI Controls (n=23) | Elite Athletes (n=40) |
|---------------------------|----------------------------|-----------------------------|--------------------------|
| Phylum | | | |
| Proteobacteria | 5.602±1.106 | 5.554±1.160 | 7.079±1.098 |
| Bacteroidetes | 51.992±2.873 | 58.249±3.387† | 44.346±1.967 |
| Firmicutes | 39.910±2.747 | 34.261±2.586† | 46.690±1.788 |
| Lentisphaerae | 0.424±0.130 | 0.600±0.798 | 0.229±0.099 |
| Tenericutes | 0.502±0.523 | 0.031±0.016 | 0.267±0.079 |
| Actinobacteria | 0.990±0.237# | 0.833±0.306 | 0.817±0.181 |
| Cyanobacteria | 0.131±0.055 | 0.011±0.010 | 0.029±0.015 |
| Verrucomicrobia | 0.136±0.051 | 0.067±0.046 | 0.042±0.012 |
| RF3 | 0.141±0.062 | 0.027±0.022 | 0.040±0.033 |
| Spirochaetes | 0.003±0.002 | 0.000±0.000 | 0.094±0.057‡ |
| Fusobacteria | 0.004±0.003 | 0.000±0.000 | 0.003±0.002 |
| Candidate Division TM6 | 0.000±0.000 | 0.000±0.000† | 0.018±0.015‡ |
| Planctomycetes | 0.000±0.000 | 0.000±0.000† | 0.013±0.008‡ |
| Deferribacteres | 0.000±0.000 | 0.000±0.000 | 0.008±0.004 |
| Chlamydiae | 0.000±0.000 | 0.000±0.00† | 0.014±0.012‡ |
| Deinococcus Thermus | 0.000±0.000 | 0.000±0.000 | 0.008±0.006 |
| Candidate Division OD1 | 0.000±0.000 | 0.000±0.000 | 0.002±0.001 |
| Chloroflexi | 0.000±0.000 | 0.000±0.000† | 0.020±0.016‡ |
| Acidobacteria | 0.000±0.000 | 0.000±0.000 | 0.001±0.002 |
| Gemmatimonadetes | 0.000±0.000 | 0.000±0.000 | 0.001±0.001 |
| Fibrobacteres | 0.000±0.000 | 0.000±0.000 | 0.001±0.002 |
| Candidate Division TM7 | 0.000±0.000 | 0.000±0.000 | 0.005±0.004 |
| Other | 0.164±0.044 | 0.367±0.295 | 0.270±0.033 |

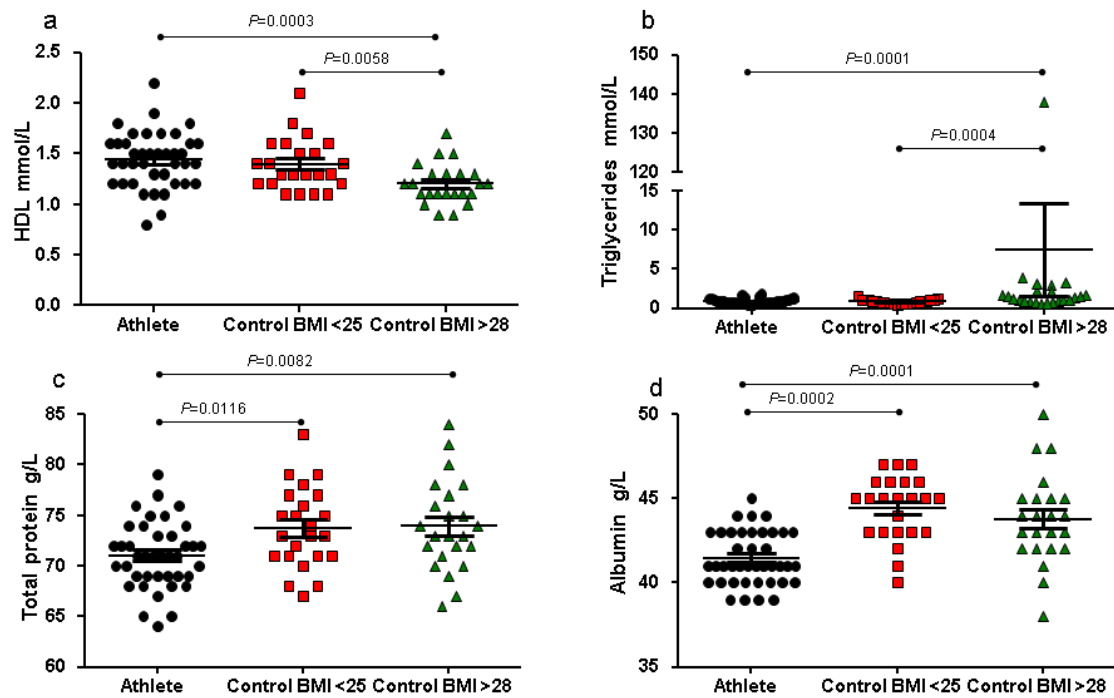
Statistical significance was determined using kruskal wallis. p value ≤ 0.05 † between high BMI controls and athletes, ‡ between low BMI controls and athletes, # between high BMI and low BMI controls. Values are mean percentage read number \pm SEM.



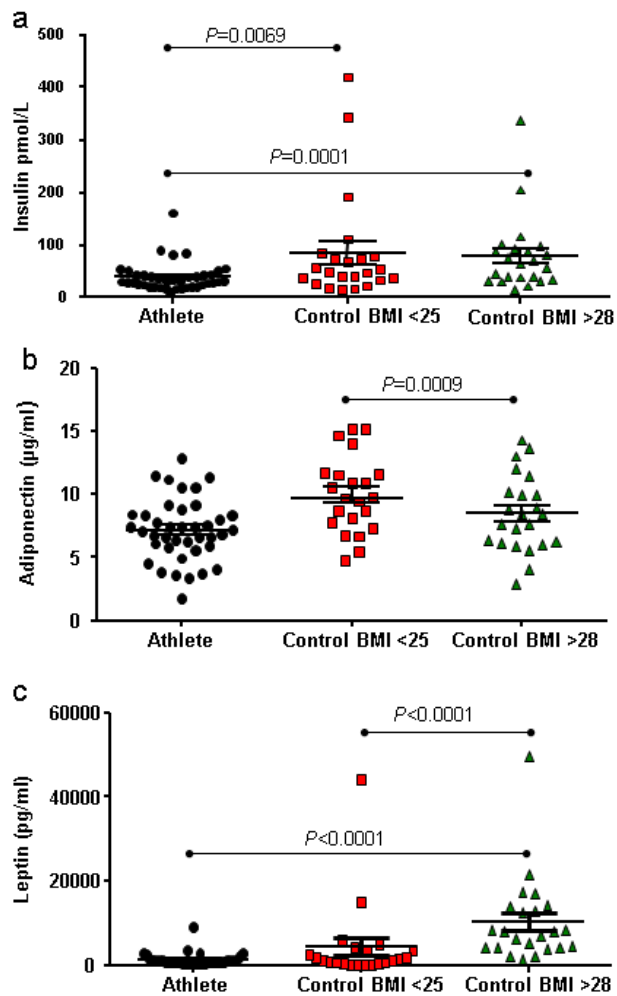
Supplementary figure 1. Cytokine levels vary between athletes and control cohorts. Scatter plot of (A) Interlukin-1 β ; (B) Interlukin-8; (C) Interlukin-6; and (D) TNF- α . (n=40 Athletes; n=23 low BMI control and n=23 high BMI control). Data expressed as mean value \pm SEM. Statistical significance was determined by Mann-Whitney for each pair-wise comparison. Note one low BMI control was removed from these graphs due to elevated cytokine values associated with completion of a marathon prior to blood collection. Removal of this control changes the p value between athletes and low BMI controls to p=0.0051 for IL-8. Two new significant results also appear between athletes and low BMI controls in IL-6 p=0.0373 and low BMI controls and high BMI controls in TNF- α p=0.0121.



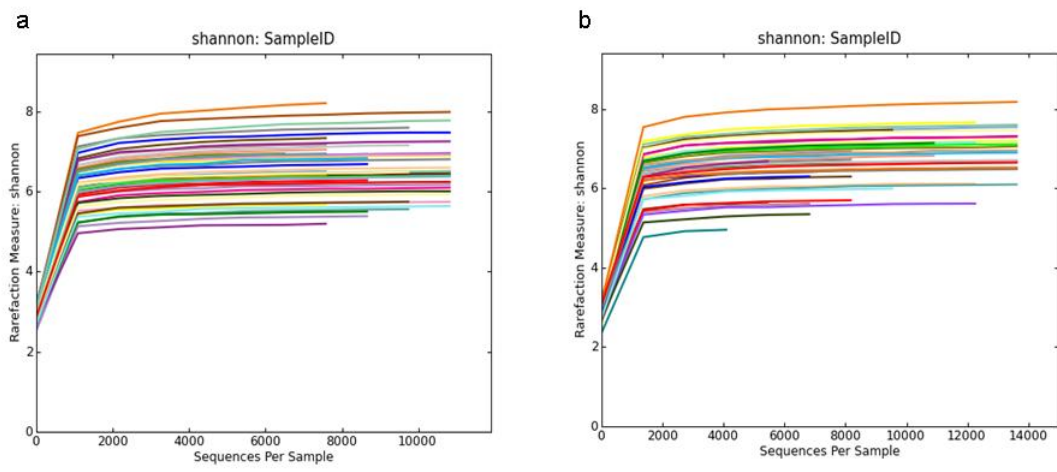
Supplementary figure 2. Differences in the blood biochemistry of athletes and controls. Scatter plot of (A) creatinine; (B) sodium; (C) potassium; (D) urea; (E) AST; and (F) YGT. (n=40 Athletes; n=23 low BMI control and n=23 high BMI control). Data expressed as mean value \pm SEM. Statistical significance was determined by Mann-Whitney for each pair-wise comparison.



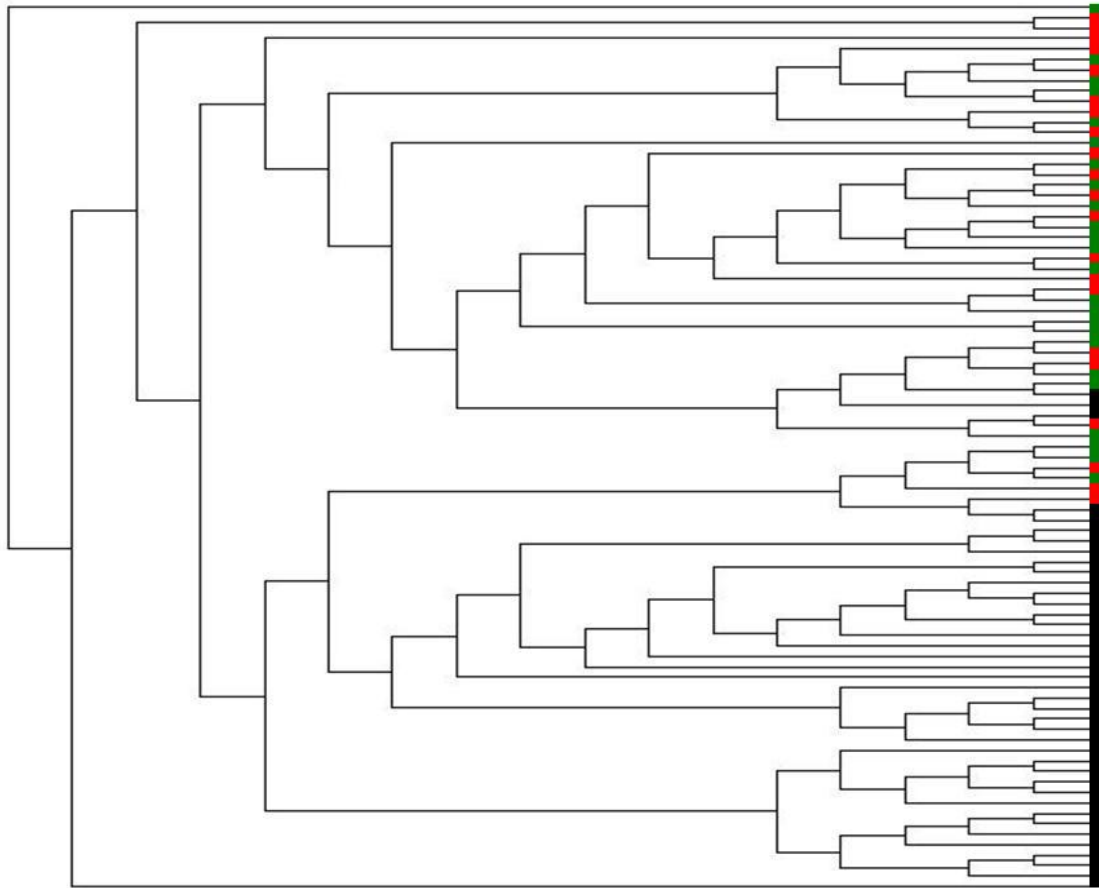
Supplementary figure 3. Study participants blood biochemistry. Scatter plot of (A) HDL; (B) triglycerides; (C) total protein; and (D) albumin. (n=40 Athletes; n=23 low BMI control and n=23 high BMI control). Data expressed as mean value \pm SEM. Statistical significance was determined by Mann-Whitney for each pair-wise comparison.



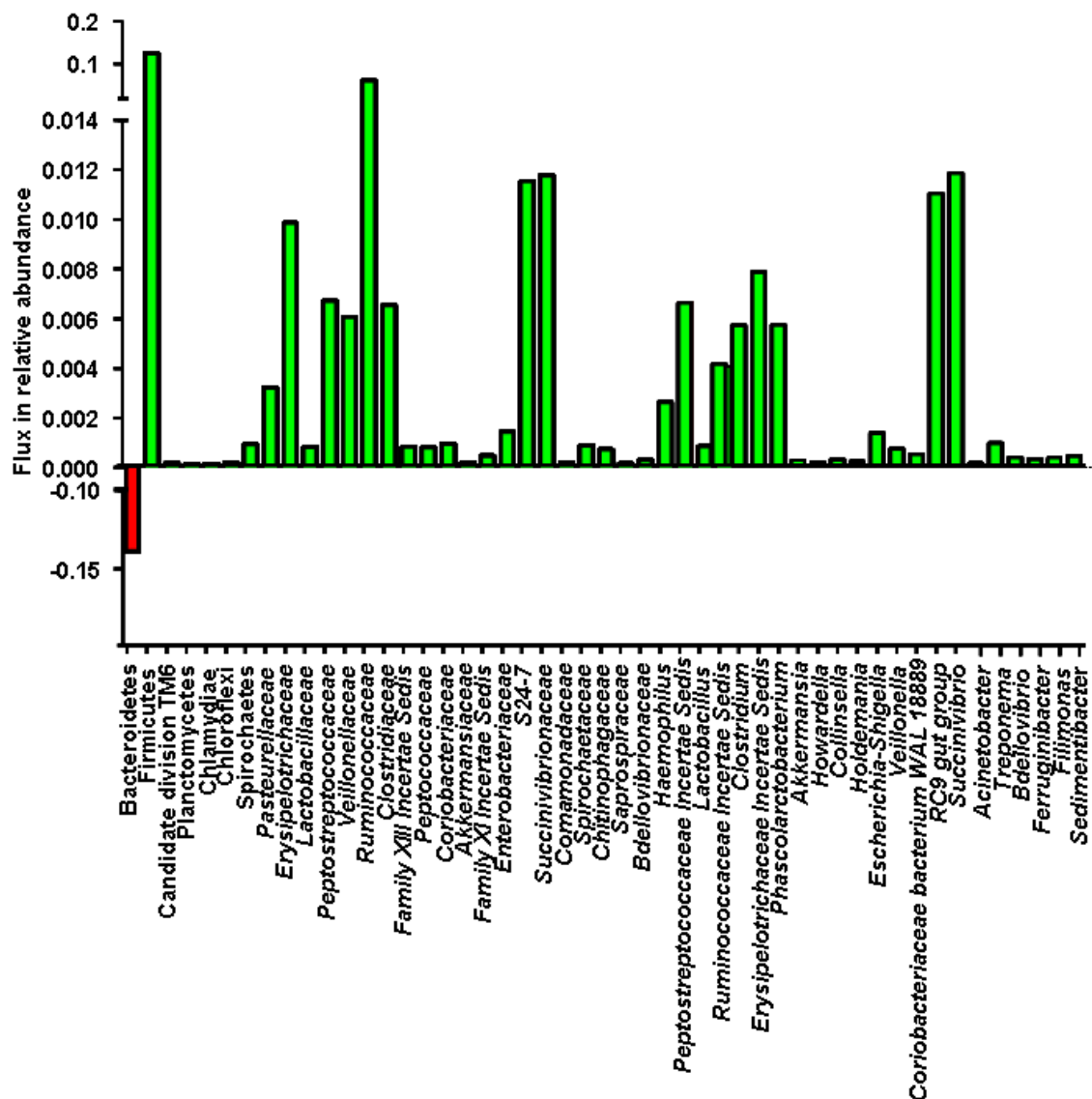
Supplementary figure 4. Significantly lower leptin and insulin levels are evident in elite athletes. Scatter plot of (A) insulin; (B) adiponectin and (C) leptin across the three cohorts. (n=40 Athletes; n=23 low BMI control and n=23 high BMI control). Data expressed as mean value \pm SEM. Statistical significance was determined by Mann-Whitney for each pair-wise comparison.



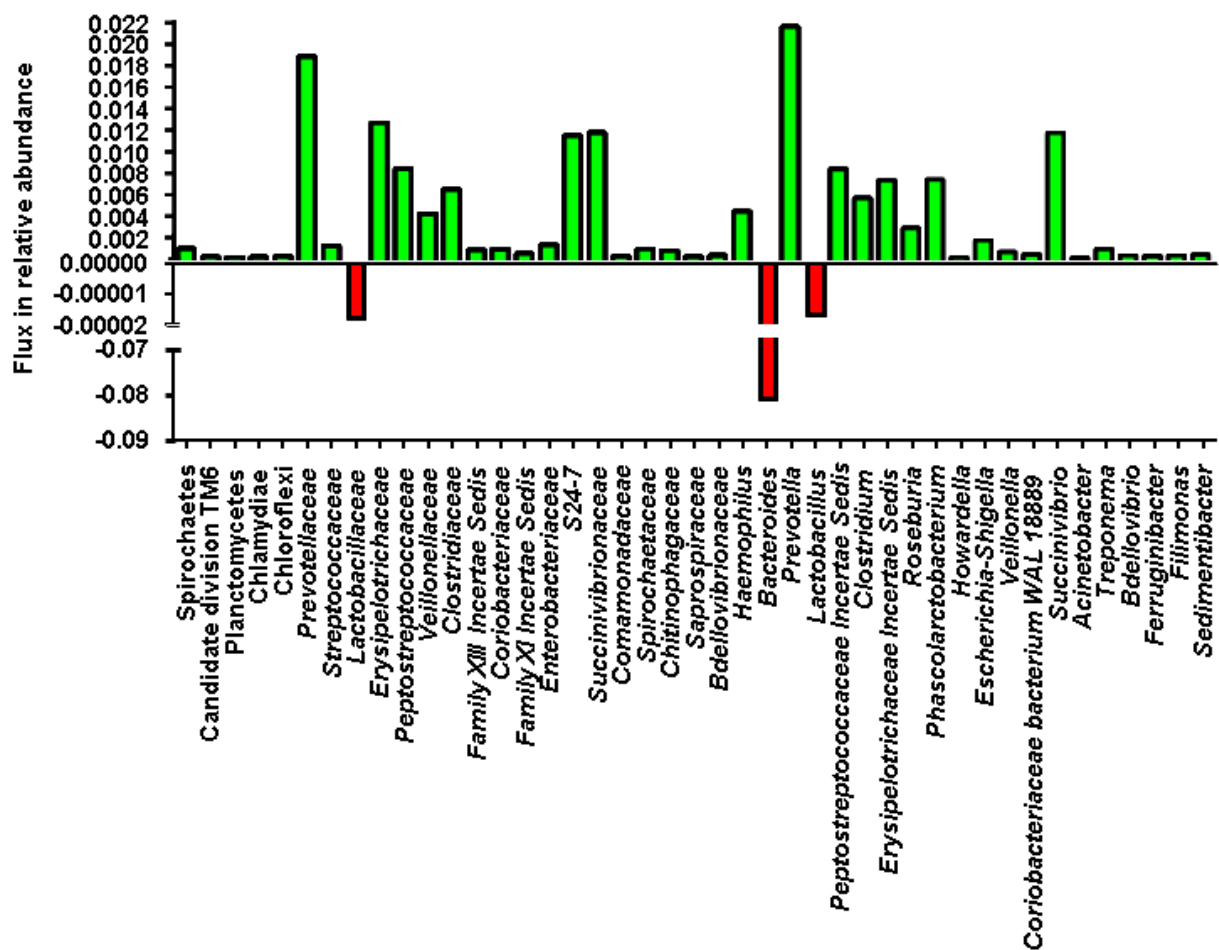
Supplementary figure 5. Rarefaction curves for (A) controls and (B) athletes at 97% similarity levels. Amount of operational taxonomic units (OTU's) found as a function of the number of sequence tags sampled.



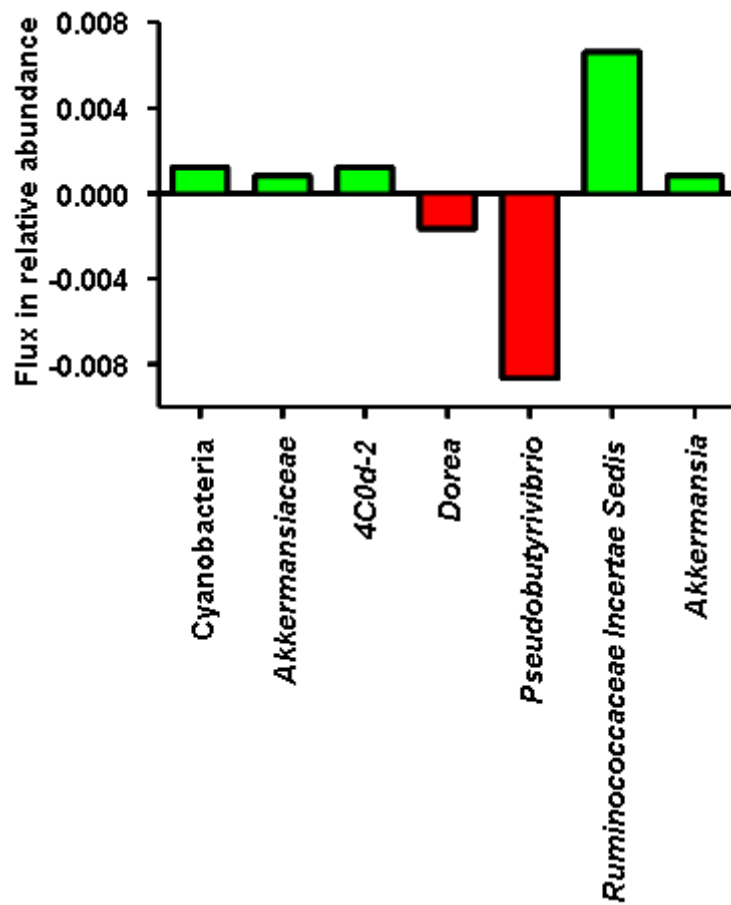
Supplementary figure 6. Unweighted pair group method with arithmetic mean (UPGMA) tree of all subjects. Subject colour coding: black, elite athletes; green, high BMI controls; and red, low BMI controls.



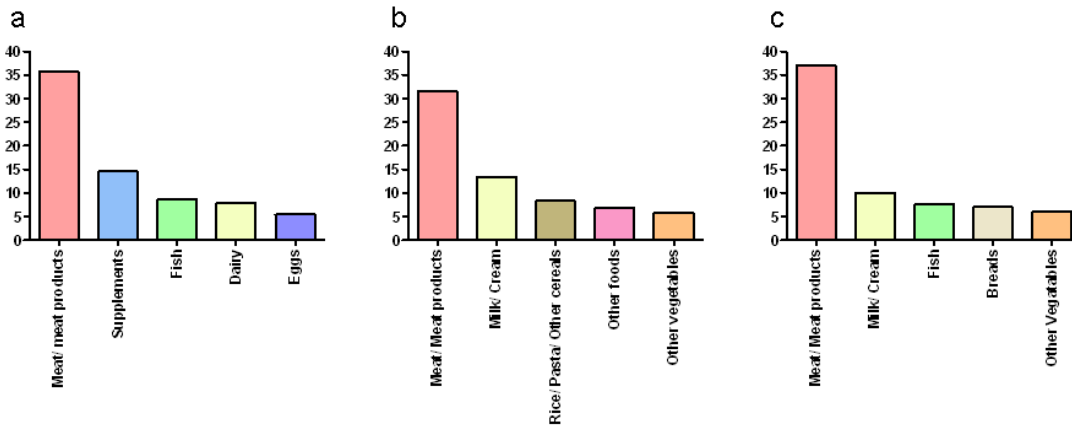
Supplementary Figure 7. Relative change in the abundance of selected taxa in athletes relative to high BMI controls. Only taxa with significant differences in population numbers are illustrated. Flux was calculated by subtracting the relative abundance of taxa in high BMI controls from the relative abundance in athlete.



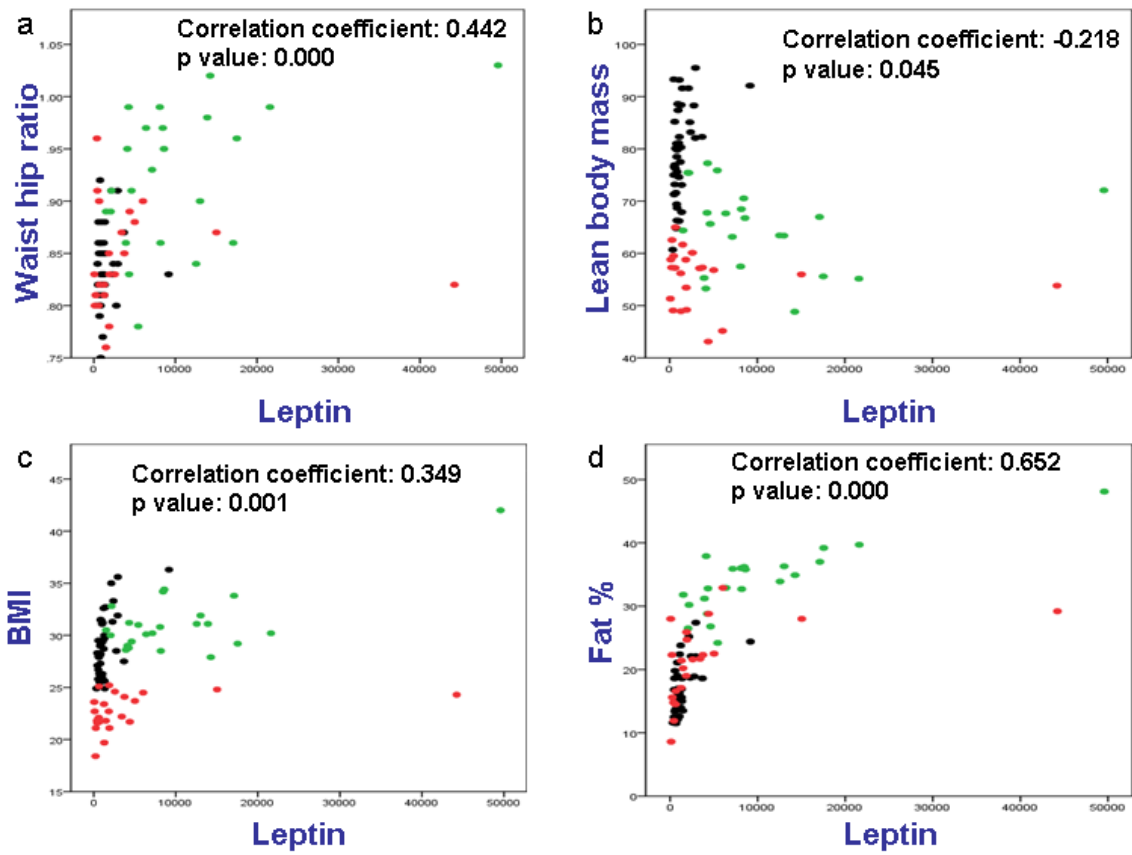
Supplementary Figure 8. Relative change in the abundance of selected taxa in athletes relative to low BMI controls. Only taxa with significant differences in population numbers are illustrated. Flux was calculated by subtracting the relative abundance of taxa in low BMI controls from the relative abundance in athletes.



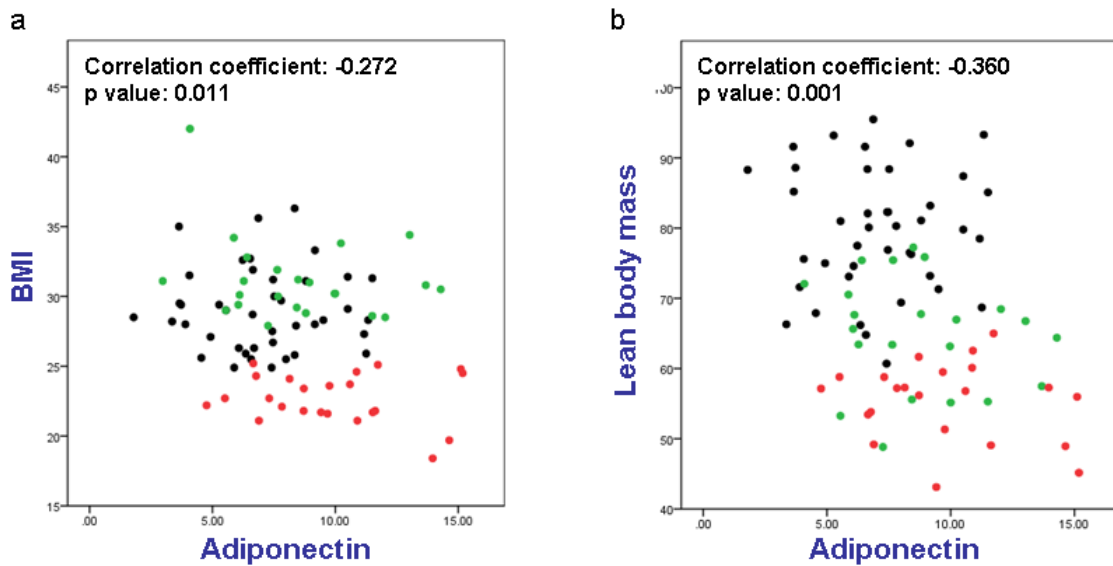
Supplementary Figure 9. Relative change in the abundance of selected taxa in low BMI controls relative to high BMI controls. Only taxa with significant differences in population numbers are illustrated. Flux was calculated by subtracting the relative abundance of taxa in high BMI controls from the relative abundance in low BMI controls.



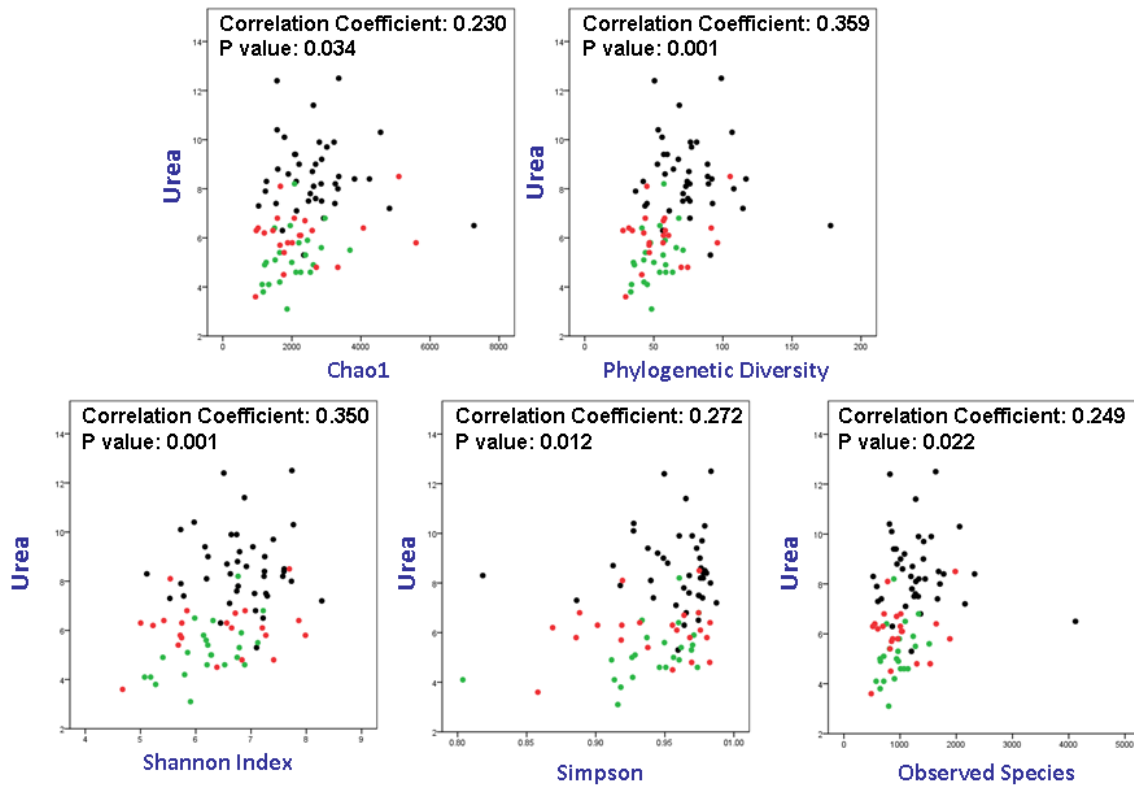
Supplementary figure 10. Top 5 food groups contributing to protein in all subjects. A, athletes: b, low BMI controls and c, high BMI controls.



Supplementary figure 11. Leptin levels positively correlate with percentage body fat, BMI, waist:hip ratio and negatively correlate with lean body mass. Subject colour coding: black, elite athletes; green, high BMI controls; and red, low BMI controls.



Supplementary figure 12. Adiponectin levels negatively correlate with BMI and lean body mass. Subject colour coding: black, elite athletes; green, high BMI controls; and red, low BMI controls.



Supplementary figure 13. Urea levels positively correlates with α diversity. Subject colour coding: black, elite athletes; green, high BMI controls; and red, low BMI controls