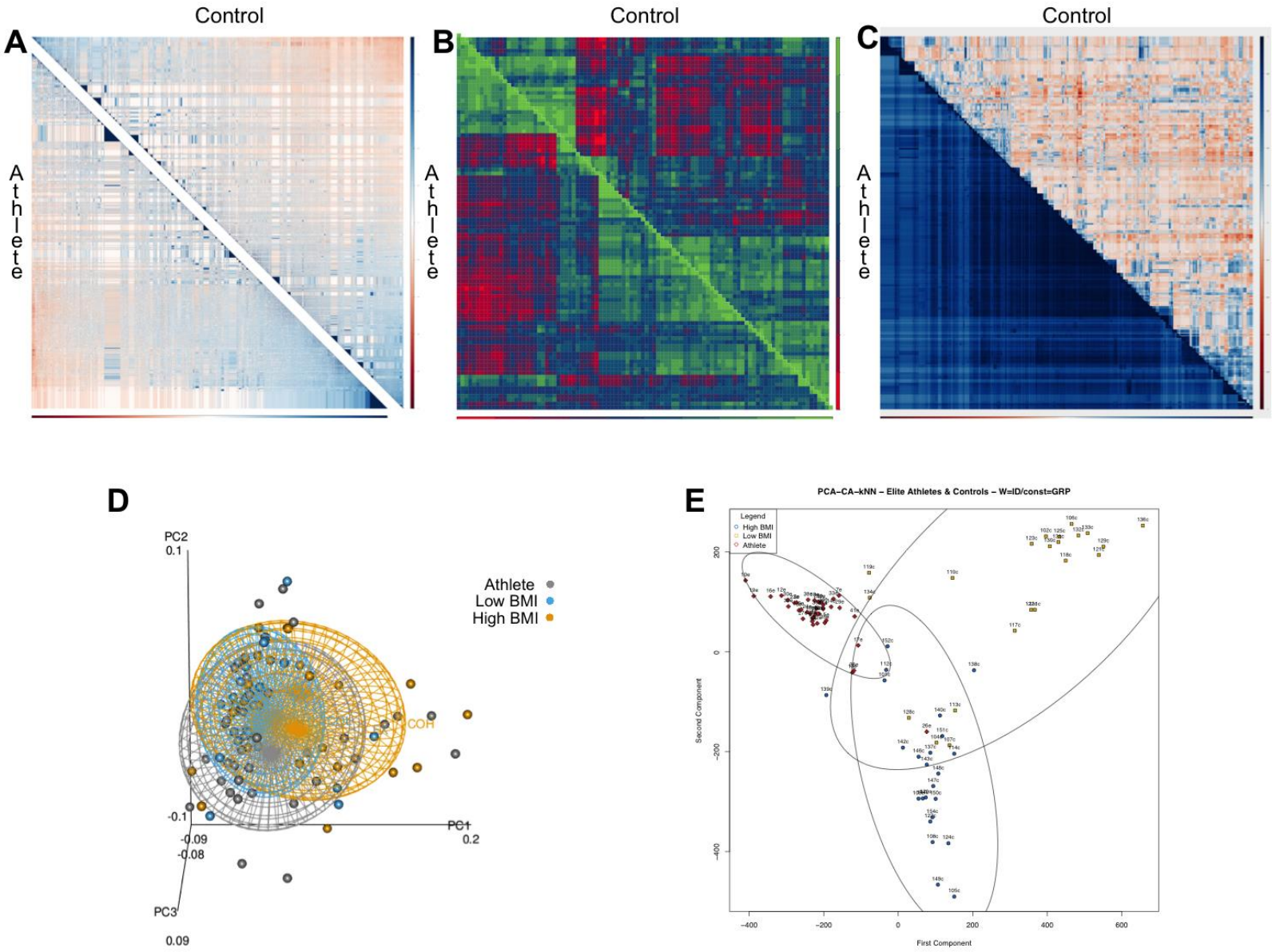
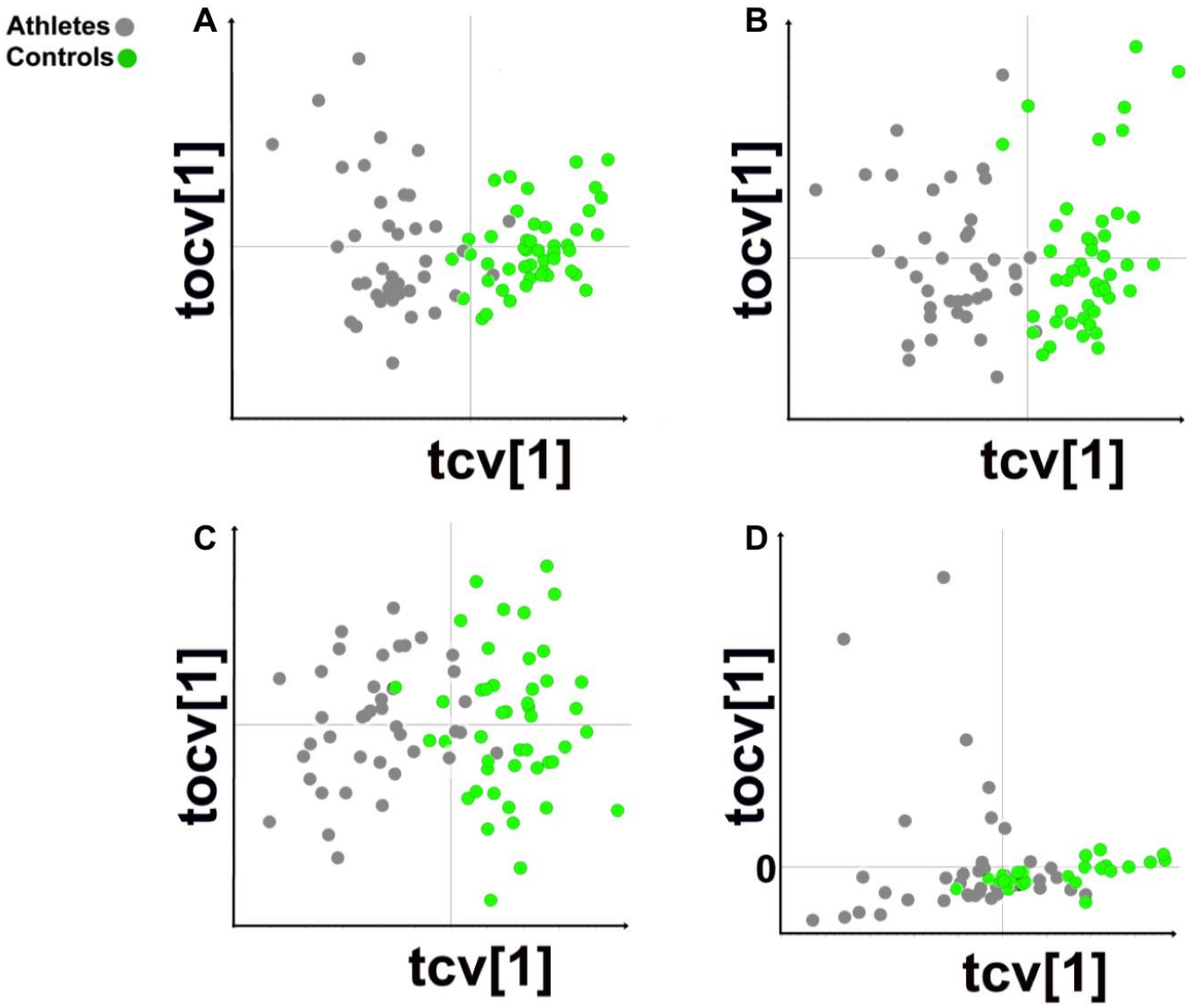
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Title	The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level
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Supplementary Figure 1 | Comparison of metabolic pathway composition between groups



Supplementary Figure 2 | OPLS-DA cross-validated scores plots comparing athletes to controls

SCFA	Subjects	n	Mean (uM)	SD	Median (uM)	IQR	Mann-Whitney U test p value (2-tailed)	pFDR (BH corrected)
Acetate	Athlete	40	5822.3	1169	5883.5	1628	0.00002	0.00013
	Control	23	4377	1200.7	4092.1	1704		
Propionate	Athlete	40	3419.3	909.8	3359.3	1231	0.00000	0.00000
	Control	23	2077.1	729.4	1910.5	1188		
Butyrate	Athlete	40	5561.5	2449.1	5234.3	2051	0.00003	0.00013
	Control	23	3397	1963.8	3091.2	1639		
Valerate	Athlete	40	429.4	222.1	421.5	237	0.00676	0.01120
	Control	23	283.6	189.9	302.9	260		
Isobutyrate	Athlete	40	277.9	170.4	255.6	175	0.18338	0.21800
	Control	23	217.3	120.6	176.1	180		
Isovalerate	Athlete	40	407.8	270.2	367.6	267	0.36746	0.36500
	Control	23	344.8	207.1	278.5	356		

Supplementary Table 5 | Short Chain Fatty Acid concentrations and statistical analysis.

		Lean mass	Protein	Fibre	Simpson diversity	Shannon diversity	PD whole tree diversity
Acetic acid	Spearman's Correlation Coefficient	0.54					
	Sig. (2-tailed)	0					
Propioninc acid	Spearman's Correlation Coefficient	0.6	0.45	0.34			
	Sig. (2-tailed)	0	0.0003	0.0069			
Butyric acid	Spearman's Correlation Coefficient	0.53	0.38	0.39			
	Sig. (2-tailed)	0	0.0024	0.0016			
Isobutyric acid	Spearman's Correlation Coefficient				0.32	0.4	0.41
	Sig. (2-tailed)				0.0116	0.0015	0.0009
Isovaleric acid	Spearman's Correlation Coefficient				0.37	0.38	0.35
	Sig. (2-tailed)				0.0035	0.0021	0.0049
Valeric acid	Spearman's Correlation Coefficient	0.33			0.3	0.37	0.33
	Sig. (2-tailed)	0.0088			0.0169	0.0035	0.0096

Supplementary Table 6 | Short Chain Fatty Acid correlation with diet and diversity.

Supplementary Figure 1 | Comparison of metabolic pathway composition between groups. Comparative correlation plots of total metabolic pathways **(A)** pathways of statistical significance **(B)** and pathways of which each is represented in at least one subject from each group **(C)**. The respective subsets of metabolic pathways are correlated separately for the athlete group (left and bottom) and all controls (right and top), after which the correlation plots are merged. The joined correlation plots illustrate distinct dynamics of pathway interrelation between athletes and controls. **(D)** Principle coordinate analysis (PCoA) of Bray-Curtis compiled distance matrix of the 98 significant pathway relative abundances. Groups show significant variation from one another (Adonis PERMANOVA P <.001). **(E)** Semi-supervised learning supplemented Principle Component Analysis (PCA-CA-kNN) of total pathway abundance values from all three groups. Outliers were detected through deviation of the defined grouping structure.

Supplementary Figure 2 | OPLS-DA cross-validated scores plots comparing athletes to controls. Models are comprised of 1 predictive (tcv[1]) and 1 orthogonal (tocv[1]) principal component. **(A)** UPLC-MS (HILIC, positive mode) urine sample analysis (R^2Y=0.85, Q^2Y=0.74), **(B)** UPLC-MS (reversed phase, positive mode) urine sample analysis (R^2Y=0.83, Q^2Y=0.73), **(C)** UPLC-MS (reversed phase, negative mode) urine sample analysis (R^2Y=0.83, Q^2Y=0.67), **(D)** UPLC-MS (HILIC, positive mode) faecal sample analysis (R^2Y=0.65, Q^2Y=0.34). Models are comprised of 1 predictive (tcv[1]) and 1 orthogonal (tocv[1]) principal component.

Supplementary Table 1 | Statistically significant metagenomic pathways. Pathways found to be statistically significant between the athlete group and both low and high BMI control groups. See attached file "Supplementary Table 1 (Sig Pathways).pdf"

Supplementary Table 2 | Significant pathways organised according to MetaCyc metabolic classification. Pathways of statistical significance were organised into metabolic classes according to the MetaCyc database. See attached file "Supplementary Table 2 (Sig Pathways in MetaCyc category).pdf"

Supplementary Table 3 | List of significantly associated metabolites whose excretion discriminates between athletes and controls when using 1H-NMR metabolic profiling models of urine (Figure 2C) and faecal water (Figure 2D). Lists the metabolite name, significant chemical shift values and multiplicity, LoA and direction of association (↑ indicates higher excretion in the athletes group, ↓ indicates lower excretion in the athletes group) for urine and faecal biofluids. See attached file "Supplementary Table 3 - NMR OPLS-DA IDs.pdf"

Supplementary Table 4 | List of significantly associated metabolites whose excretion discriminates between athletes and controls when using LC-MS metabolic profiling models of urine (Supplementary figure 2A-D). Lists the metabolite name, liquid chromatography (LC)-platform, mass charge ratio (m/z), MSMS fragments, retention time (RT), level of assignment (LoA) and direction of association (↑ indicates higher excretion in the athletes group, ↓ indicates lower excretion in the athletes group) in urine. See attached file "Supplementary Table 4 - MS OPLS-DA IDs (level 2 only).pdf"

Supplementary Table 5 | Short Chain Fatty Acid concentrations and statistical. analysis. Concentrations of SCFAs detected in faecal water samples. Statistical testing was performed on concentrations, comparing the athlete and control group.

Supplementary Table 6 | Short Chain Fatty Acid correlation with diet and diversity. SCFA concentrations correlated to measures of gut microbial diversity and participant dietary and body composition measurements.

Supplementary Table 7 | Short Chain Fatty Acid correlation with taxa. Statistically significant correlations between faecal water derived concentrations of SCFAs and 16S identified gut bacteria. See attached file "Supplementary Table 7 (SCFA v Taxa).pdf"

Supplementary Table 8 | Short Chain Fatty Acid correlation with metagenomic pathways. List of statistically significant correlations between faecal water derived concentrations of SCFAs and metagenomic pathways. See attached file "Supplementary Table 8 (SCFA v Metagenomic).pdf"

Supplementary Table 9 | Correlation of gut bacterial taxa with metabolites. Statistically significant correlations of metabolites derived from both urine and faecal water to genera of gut microbiota. See attached file "Supplementary Table 9 (Metabolite v Taxa).pdf"