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Gut *Prevotella copri* abundance linked to elevated post-exercise inflammation

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Abstract

Purpose: This study aimed to examine the linkage between gut microbiome taxa and exercise-induced inflammation.
Methods: Twenty-five cyclists provided 4 stool samples during a 10-week period and cycled vigorously for 2.25 h at 67% maximal oxygen uptake (VO_{2max}) in a laboratory setting. Blood samples were collected pre- and post-exercise, with additional samples collected at 1.5-h, 3-h, and 24-h post exercise. Primary outcomes included stool microbiome composition and alpha diversity via whole genome shotgun (WGS) sequencing (averaged from 4 stool samples) and a targeted panel of 75 plasma oxylipins. A total of 5719 taxa were identified, and the 339 that were present in more than 20% of stool samples were used in the analysis. Alpha diversity was calculated by evenness, and the Analysis of Composition of Microbiomes (ANCOM) differential abundance analysis was performed using Quantitative Insights Into Microbial Ecology-2 (QIIME2). A composite variable was calculated from 8 pro-inflammatory oxylipins generated from arachidonic acid (ARA) and cytochrome P-450 (CYP).
Results: ARA-CYP oxylipins were significantly elevated for at least 3-h post-exercise ($p < 0.001$); they were strongly and positively related to *Prevotella copri* (*P. copri*) abundance ($R^2 = 0.676$, $p < 0.001$) and negatively related to gut microbiome alpha diversity ($R^2 = 0.771$, $p < 0.001$).
Conclusion: This analysis revealed for the first time a novel, positive relationship between gut microbiome *P. copri* abundance in cyclists and post-exercise pro-inflammatory oxylipins. These data demonstrate that about two-thirds of the wide variance in inflammation following prolonged and intensive exercise is largely explained by the abundance of a single gut bacterial species: *P. copri.*

Keywords: Gut microbiome; Exercise; Inflammation; Oxylipins

1. Introduction

Prolonged and intense cycling and running induce significant inflammation for several hours post-exercise.[1–3] Commonly measured inflammation biomarkers include cytokines, acute phase and other innate immune system proteins, blood leukocyte subset counts, and oxylipins. The post-exercise inter-individual inflammation response variance is large.[1] For example, in a recent study of 25 cyclists who cycled vigorously for 2.25 h, post-exercise elevations in proinflammatory oxylipins generated from arachidonic acid (ARA) and cytochrome P-450 (CYP) varied 20-fold.[2] Runners who completed the 160-km Western States Endurance Run experienced increases in C-reactive protein (CRP) that varied from 0 to 95 mg/dL and in interleukin-6 (IL-6) from 0 to 800 pg/mL.[1]

Underlying factors explaining the highly variable post-exercise inflammation response are poorly understood. Muscle damage varies widely in runners after marathons and ultramarathons, and changes in related biomarkers such as creatine kinase have been modestly but inconsistently related to post-exercise changes in IL-6 and CRP.[1] There is an increasing awareness that the gut microbiome plays a significant role in systemic inflammation.[4,5] The gut microbiome helps regulate immune responses through bidirectional interactions, and certain gut bacteria and related byproducts can trigger

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inflammation. Gut dysbiosis can increase intestinal permeability and the release of endotoxins into the bloodstream that induces inflammation.[4,5] Correlations between inflammatory cytokines, such as IL-6 and tumor necrosis factor-alpha (TNF-α), and specific species of gut microbiota have been established.[6] Whether or not the gut microbiome is related to varying inflammation responses to acute stressful exercise workloads has not been reported previously.

This study examined the potential linkage between the gut microbiome composition and post-exercise inflammation. Stool microbiome composition from 25 cyclists was assessed via whole genome shotgun (WGS) sequencing and related to changes in post-exercise plasma oxylipin levels following 2.25 h of intensive cycling. Recent animal and human data support a key role for gut bacteria in the generation of oxylipins functioning as lipid mediators of inflammation.[7,8] Oxylipins are oxidized during physiological stress from polyunsaturated fatty acids (PUFAs) including linoleic acid (LA), α-linolenic acid (ALA), ARA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Oxylipin production from PUFA oxidation occurs primarily through cyclooxygenase (COX), lipoxygenase (LOX), and CYP enzyme systems.[3] Oxylipins are generated transiently during stressful levels of exercise, are sensitive upstream indicators of inflammation, and can be lowered through nutrition-based interventions, including carbohydrate and flavonoid supplementation.[3] We hypothesized that gut microbiome alpha diversity and specific bacterial taxa would partially explain the wide variance in post-exercise increases in proinflammatory oxylipins. Due to the exploratory nature of this seminal analysis, relationships between 339 taxa from WGS sequencing and 75 oxylipins were probed using principal component analysis (PCA), cluster analysis, and linear regression by the least squares method between all possible predictor and response combinations.

2. Methods

2.1. Study participants

The data for this paper come from a secondary analysis of gut microbiome (from 4 stool samples that did not vary from the intervention) and plasma oxylipin data (placebo trial only) from a previously published clinical trial.[2] In this study, male and female cyclists ($n = 25$ total) were invited to take part if they met the inclusion criteria: 18−55 years of age, is capable of cycling 2.25 h in a laboratory setting at close to 70% maximal oxygen consumption rate (VO_{2max}) and is willing to avoid supplements, herbs, and medications with a potential to influence inflammation (e.g., non-steroidal anti-inflammatory drugs (NSAIDs)) for 2 weeks before and during the 10-week study period. No restrictions were placed on yogurt or probiotics intake. Participants agreed to maintain normal diet and exercise habits during the study period. Each of the study participants were screened for the absence of coronavirus disease 2019 and other respiratory illnesses. None of the subjects reported antibiotic use for 2 weeks before or during the 10-week period during which 4 stool samples were collected. All participants entered and completed the study within a concise 3-month period (July−September 2022). The lab temperature and relative humidity during the 2.25-h cycling bouts (each starting at 7:00 a.m.) averaged 21.3°C ± 0.4°C and 46% ± 1.1% (mean ± standard error of mean (SE). During the 3-day period *prior to* the 2.25-h cycling session, study participants agreed to taper exercise training and ingest a moderate-carbohydrate diet using a food list restricting high fat foods and visible fats. Participants were given a complete orientation to the study and then voluntarily signed the informed consent. Study procedures were approved by the Appalachian State University's Institutional Review Board (IRB 22-0114).

2.2. Study design

As reported previously,[2] height and body weight were assessed in a pre-study lab session, with body composition measured using the BodPod system (COSMED, Rome, Italy). Study participants were tested for maximal aerobic capacity (VO_{2max}) during the pre-study lab session using a graded, cycling test with the Lode cycle ergometer (Lode B.V., Groningen, the Netherlands) and the COSMED cardiopulmonary exercise test (CPET) metabolic cart (COSMED). Stool kits were supplied, and participants provided 4 stool samples during a 10-week period using the OMNIgene•GUT OMR-200 kit (DNA Genotek, Ontawa, Canada). Stool samples were collected at baseline, and then again at Weeks 4, 6, and 10. Study participants reported to the test facility and turned in stool samples that had been collected the previous day. Gut microbiome composition datasets from the 4 stool samples were averaged to reduce the effect of inter-individual variation. Permutational multivariate analysis of variance analysis (PERMANOVA) for beta diversity showed no differences in the microbiome profiles between the samples ($F = 0.350$, $p = 0.993$).

Plasma oxylipins were analyzed from blood samples that were collected before and after a prolonged and intensive cycling bout. Study participants reported to the performance lab early in the morning in an overnight fasted state (>8 h). A fasting blood sample was collected. After warming up, participants cycled for 2.25 h at approximately 70% VO_{2max} while ingesting only water (3 mL/kg every 15 min). Participants cycled on their own bicycles fitted to Saris H3 direct drive smart trainers (Saris, Madison, WI, USA), with monitoring by the Zwift online training platform (Zwift, Long Beach, CA, USA) and the COSMED CPET metabolic cart. Heart rate, cycling speed, cadence, distance, power, breathing rate, ventilation, and oxygen intake were measured after 15 min and then every 30 min during the cycling session. Blood samples were collected within 5 min immediately post-exercise (0-h), and at 1.5-h, 3-h, and 24-h post-exercise. Immediately after the 1.5-h post-exercise blood sample, all subjects consumed 7 kilocalories per kilogram (kcal/kg) of body weight of a fortified nutrient beverage (Boost, Nestlé S.A., Vevey, Switzerland).

2.3. Sample analysis

Plasma aliquots were prepared from ethylenediaminetetraacetic acid (EDTA) blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and stored in a −80°C

freezer for analysis for oxylipins.[2] Stool samples were prepared and stored in a −80°C freezer.[2]

The procedures for measuring plasma oxylipins have been described previously.[9] In brief, plasma oxylipins were analyzed using a liquid chromatography-multiple reaction monitoring mass spectrometry (LC-MRM-MS) method (Thermo Fisher Scientific, Haverhill, MA, USA). A total of 53 of 75 oxylipins detected increased significantly post-exercise, and these were grouped for statistical analysis. Four other composite variables were calculated, including 8 oxylipins generated from ARA-CYP (5,6-; 8,9-; 11,12-; and 14,15-dihydroxy-eicosatetraenoic acids (diHETrEs), and 16-, 17-, 18-hydroxy-eicosatetraenoic acids (HETEs), and the 20-HETE metabolite 20-carboxy-arachidonic acid (20-coohAA)), a subset of 4 oxylipins generated from ARA-CYP and soluble epoxide hydrolase (sEH) (5,6-; 8,9-; 11,12-; and 14,15-diHETrEs), 2 abundant oxylipins generated from linoleic acid (LA) and CYP (9,10- dihydroxy-9Z-octadecenoic acid (DiHOME) and 12,13-DiHOME), and 2 abundant oxylipins generated from LA and lipoxygenase (LOX) (9-hydroxy-octadecadienoic acid (HODE) and 13-HODE).

The procedures for the gut microbiome analysis have been described in detail with 10 supporting references.[2] Stool samples were assayed at the Microbiome Core of the University of North Carolina at Chapel Hill's School of Medicine (https://www.med.unc.edu/microbiome/). In brief, DNA was isolated and purified from the stool samples, and then assessed using Illumina WGS sequencing (Illumina, San Diego, CA, USA). Sequencing output from the Illumina NextSeq 4000 platform was converted to fastq format and demultiplexed using Illumina Bcl2Fastq 2.20.0 (Illumina). Quality control of the demultiplexed sequencing reads was verified by FastQC (Babraham Institute, Cambridge, UK). Adapters were trimmed using Trim Galore (Babraham Institute, Cambridge, UK). The resulting paired-end reads were submitted to Kraken2 for taxonomic classification. An estimate of taxonomic composition including host was produced from these results using Bracken 2.5. Alpha diversity was measured by evenness, and Analysis of Compositions of Microbiomes (ANCOM) differential abundance analysis was performed using Quantitative Insights Into Microbial Ecology-2 (QIIME2) (https://qiime2.org). Alpha diversity analysis was performed on data rarefied to a uniform sampling depth of 5000 reads. Diversity analysis and differential abundance analysis were performed on features that exceeded a total abundance of 5000 reads, corresponding to on average 0.375% relative abundance and prevalence of occurring in at least 20% of the samples.

2.4. Statistical analysis

Data are expressed as mean ± SE. Post-exercise increases in oxylipins were analyzed using SPSS (Version 28.0; IBM Corp., Armonk, NY, USA). *Post hoc* analyses were conducted using paired *t* tests comparing time point contrasts. An α level of $p \leq 0.0125$ was used after Bonferroni correction for 4 multiple tests.

Other statistical analyses were performed in RStudio Version 2023.12.1+402[10] using R Version 4.4.[11] Initial testing involved principal component analysis (PCA) and cluster analysis of the microbiota taxa (average from 4 stool samples) and linear regression by least squares method performed between all possible predictor and response combinations. A data frame was generated that contained correlation coefficients and R^2 values on the different crosses between mean subject bacterial species abundances, alpha diversity, and 0 to 3-h post-exercise oxylipin data. Power calculations for correlation and linear regression were done in R using the package "pwr" Version 1.3-0, and were 0.968 and 0.776, respectively, at $p = 0.001$. The effect size used in the calculation was equal to 1.

Both the response and predictor variable lists were subjected to $\log_{10}$ and Box-Cox (BC) transformations. Shapiro-Wilks tests were used to assign a boolean for the Gaussian distribution of the raw and transformed predictor and response data. The data were considered to be Gaussian if the *p* value was greater than 0.05 and the test statistic was at least 0.65. BC were performed using MASS Version 7.3.60.2.[12]

Person's correlation, Spearman's correlation, and simple linear regression were performed on the models with 5 dataset pairings. The models were first filtered based on Pearson's correlation, and then by the R^2 value that was produced for each model by simple linear regression. The model was considered meaningful if the correlation coefficient and R^2 values were at least 0.65. Spearman's correlation was not used in this analysis due to its mathematical partisanship to monotonic relationships and its weakness as a useful measure for linearity between a predictor and response.

Potential outliers in the raw and transformed data were elucidated using ±1.5 × inter-quartile range (IQR) exclusion criterion. Outlier confirmation was evaluated by within-subject comparison of the data from the 4 stool samples with ±50% of the standard deviation of the mean of all 100 samples, as well as percent rank and *z*-score.

The significant models were evaluated further using *k*-fold cross validation (CV).[13] CV was used to measure the predictive ability of the model in a random sample evaluated using root mean square error (RMSE) and mean absolute error (MAE). RMSE and MAE are expressed as percentage of the range. Models with RMSE and MAE values that were less than or equal to 10% of the range were considered to have high predictability. Training data were calculated from a random sampling of 80% of the original data; the remaining 20% was used for testing. Training data and the model for CV were produced using caret Version 6.0.94.[13]

To examine the effect of sample size on the models, a dataset with an inflated sample size of 1000 subjects was created using faux Version 1.2.1.[14] The same process of data transformation and CV were used to assess the significant models' performance in a larger population with similar data structure.

3. Results

Characteristics for $n = 25$ study participants ($n = 17$ males and $n = 8$ females) have been described previously.[2] Briefly,

male and female cyclists were of similar age (43.2 ± 2.2 years and 41.8 ± 2.9 years, respectively) and percent body fat (22.1% ± 1.7% and 26.0% ± 3.6%, respectively). Aerobic power, or VO_{2max} (mL/kg/min), was higher in the male *vs.* female cyclists (46.2 ± 2.1 and 37.4 ± 2.1, respectively; $p < 0.05$). The average percent of maximum heart rate (78.7% ± 1.7%) and VO_{2max} (66.6% ± 2.3%) during the cycling trial did not differ significantly between sex groups. Additionally, the patterns of change over time, or interaction effects, did not differ between the male and female cyclists for total plasma oxylipins and gut microbiome alpha diversity (both $p > 0.05$). Thus, this analysis is presented for all study participants combined.

Table 1 summarizes changes in the sum of 53 plasma oxylipins and 3 subgroups following the 2.25-h cycling bout. Plasma oxylipins were elevated for at least 1.5−3.0 h-post-exercise, with a return to pre-exercise levels after 24 h.

Whole genome sequencing identified 5719 taxa in the stool samples, with 339 present in more than 20% of stool samples. Major gut microbiome bacterial phyla and species are summarized in Fig. 1A and B. Fig. 2 compares alpha diversity in the male and female subjects (sex contrast, $p = 0.628$). Fig. 3 depicts the large subject-to-subject variance in gut bacterial species.

PCA revealed that of the identified bacterial species, *P. copri* was among the few components comprising the first principal component (PC1) (24.3%) (Fig. 4). This finding suggested that its strong potential association with inflammatory oxylipins was not an isolated finding but was indeed aligned with broader data patterns.

None of the data transformations were able to successfully normalize the data according to the Shapiro-Wilks specifications, so the results reported here are from the raw and non-transformed data. Two subjects were identified as outliers in the raw data for models with alpha diversity and *P. copri*. Another subject was a consistent outlier in the ARA-CYP and *P. copri* model in all 3 datasets. PCA was also able to isolate another subject as a potential outlier based on the structure of the data. Despite these findings, variance in the abundances of stool samples were within ±50% of the standard deviation of

Table 1
Changes in plasma oxylipin subgroups in response to 2.25 h of cycling (mean ± SE).

Variable (ng/mL)	Pre-exercise	Post-exercise			
		0 h	1.5 h	3 h	24 h
Oxylipins (total $n = 53$ oxylipins)	43.8 ± 2.6	92.9 ± 7.6[b]	65.4 ± 4.2[b]	47.4 ± 2.3[b]	44.0 ± 2.6
ARA-CYP[a] ($n = 8$ oxylipins)	6.4 ± 0.4	12.9 ± 1.9[b]	15.0 ± 2.2[b]	12.3 ± 1.5[b]	7.1 ± 0.5
LA-CYP DiHOMEs (9,10- and 12,13-)	3.7 ± 0.4	11.6 ± 1.2[b]	6.2 ± 0.7[b]	5.5 ± 0.4[b]	4.3 ± 0.6
LA-LOX HODEs (9- and 1-3)	5.5 ± 0.5	17.0 ± 1.5[b]	8.9 ± 0.8[b]	4.8 ± 0.3	6.0 ± 0.7

[a] ARA-CYP means 8 oxylipins generated from arachidonic acid and cytochrome P-450 were grouped; these included 5,6-; 8,9-, 11,12-; and 14,15-diHETrEs, 16-, 17-, 18-HETEs, and the 20-coohAA.
[b] $p < 0.0125$ relative to pre-exercise.
Abbreviations: 20-coohAA = 20-HETE metabolite 20-carboxy-arachidonic acid; ARA-CYP = arachidonic acid and cytochrome P450; diHETrEs = dihydroxy-eicosatetraenoic acids; DiHOMEs = dihydroxy-9Z-octadecenoic acids; HETEs = hydroxy-eicosatetraenoic acids; HODEs = hydroxy-octadecadienoic acids; LA, linoleic acid; LOX = lipoxygenase.

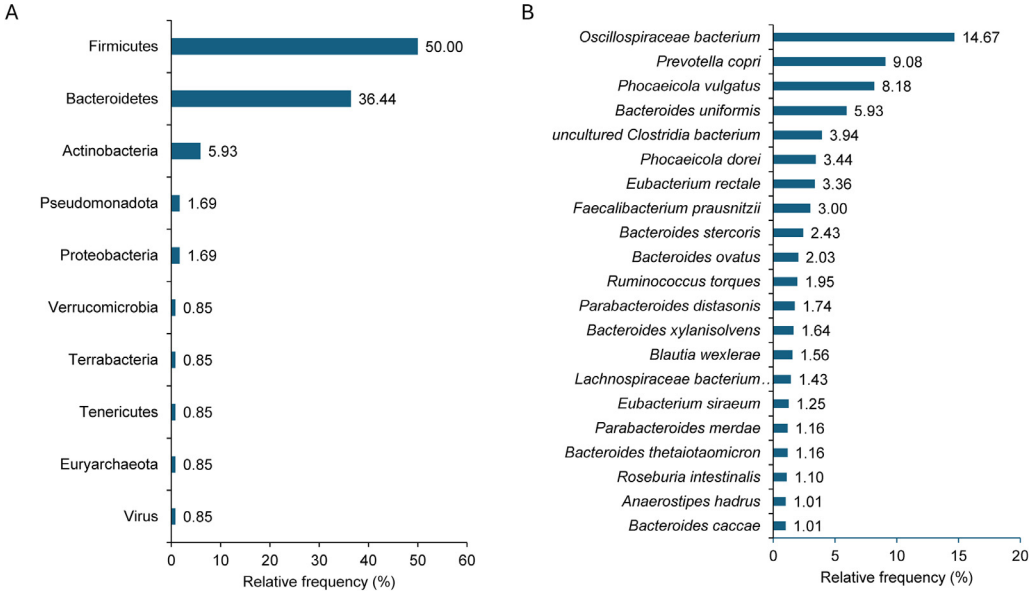

Fig. 1. (A) Phylum classification (average of 4 stool samples from 25 cyclists). (B) Relative frequency for taxa species >1.0%. Data are represented as % for 118 taxa at ≥0.05% abundance.

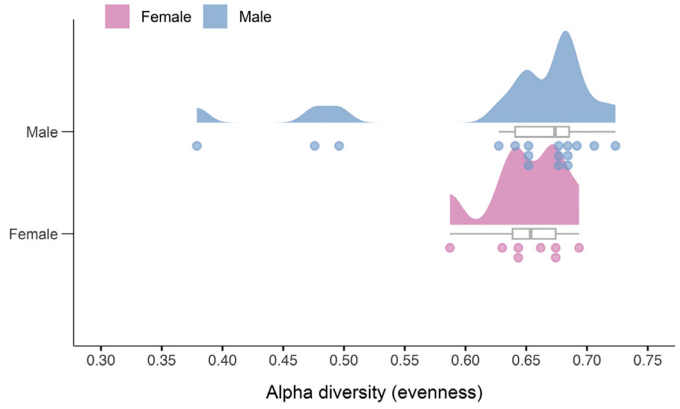

Fig. 2. Raincloud plot of gut microbiome alpha diversity (evenness) by sex ($p = 0.628$). Data were averaged from 4 stool samples collected from 25 cyclists.

the mean. Thus, none of the potential outliers were excluded from this analysis.

Every relationship between 0−3-h post-exercise oxylipin averages from Table 1 and average abundances of bacterial species from stool samples was explored with initial linear regression. These models only identified 1 significant relationship, and this was a positive and strong correlation between *P. copri* and ARA-CYP ($R^2 = 0.676$, $p < 0.001$) (Fig. 5A). There was also a significant inverse relationship observed between ARA-CYP and alpha diversity ($R^2 = 0.771$, $p < 0.001$) (Fig. 5B).

K-fold CV and inflated population analysis also supported the relationships observed in the linear model. CV results for *P. copri* and ARA-CYP ($R^2 = 0.82$, RMSE = 14.1%, MAE = 5.54%) and *P. copri* and alpha diversity ($R^2 = 0.94$, RMSE = 11.3%, MAE = 9.6%) show that these models were highly generalizable to the population studied. Despite a reduction in the R^2 value (*P. copri* and ARA-CYP: $R^2 = 0.25$; *P. copri* and alpha diversity $R^2 = 0.31$) in the inflated population study, the data still maintained their trend.

4. Discussion

The prolonged and intense cycling bout induced significant inflammation, with transient increases in 53 plasma oxylipins that were more than 2-fold higher post-exercise. Of the 3 proinflammatory oxylipin subgroups targeted in this study, ARA-CYP had the highest sustained increase throughout the 3-h exercise recovery period, similar to the results from other studies published by our research group.[3,15,16] A total of 339 taxa were present in more than 20% of stool samples, and a correlational analysis revealed that just a single bacterial species, *P. copri*, was strongly related to post-exercise plasma levels of ARA-CYP. *P. copri* abundance and post-exercise plasma ARA-CYP responses varied widely among the 25 cyclists. The finding that *P. copri* accounted for about two-thirds of the post-exercise variance in plasma ARA-CYP is a novel finding that has not been reported previously.

The gram-negative bacteria genus *Prevotella* includes several taxa, of which *P. copri* is the most prevalent species.[17,18] *P. copri* is found in about 40% of the human population and is inversely correlated with Bacteroides; in some individuals, it makes up more than 50% of the gut microbiome.[17−20] *P. copri* is more common in non-Western populations and has been associated with a high intake of fiber-rich plant foods.[19] However, a recent cross-sectional study comparing the gut microbiome compositions of vegans, vegetarians, and omnivores was unable to identify *P. copri* as a strong signature of vegetarian diets.[21] Although *P. copri* is a

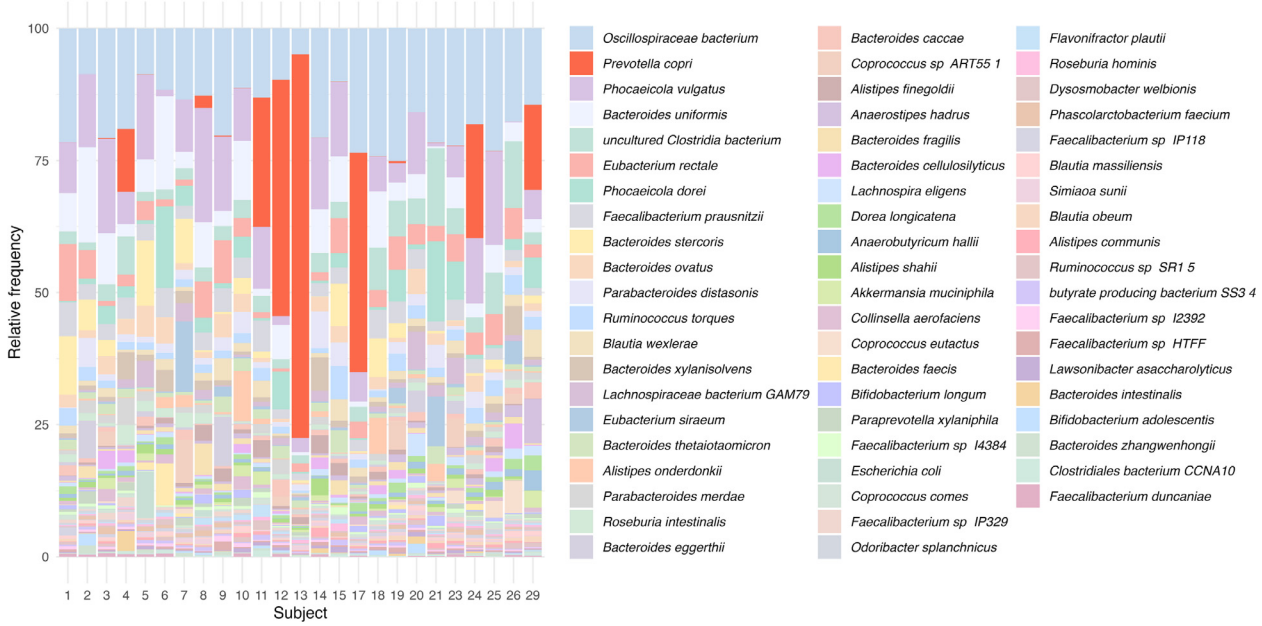

Fig. 3. Stacked bar graph highlighting subject to subject variance in *Prevotella copri* and other major gut microbiome bacterial species. Data were averaged from 4 stool samples collected from 25 cyclists.

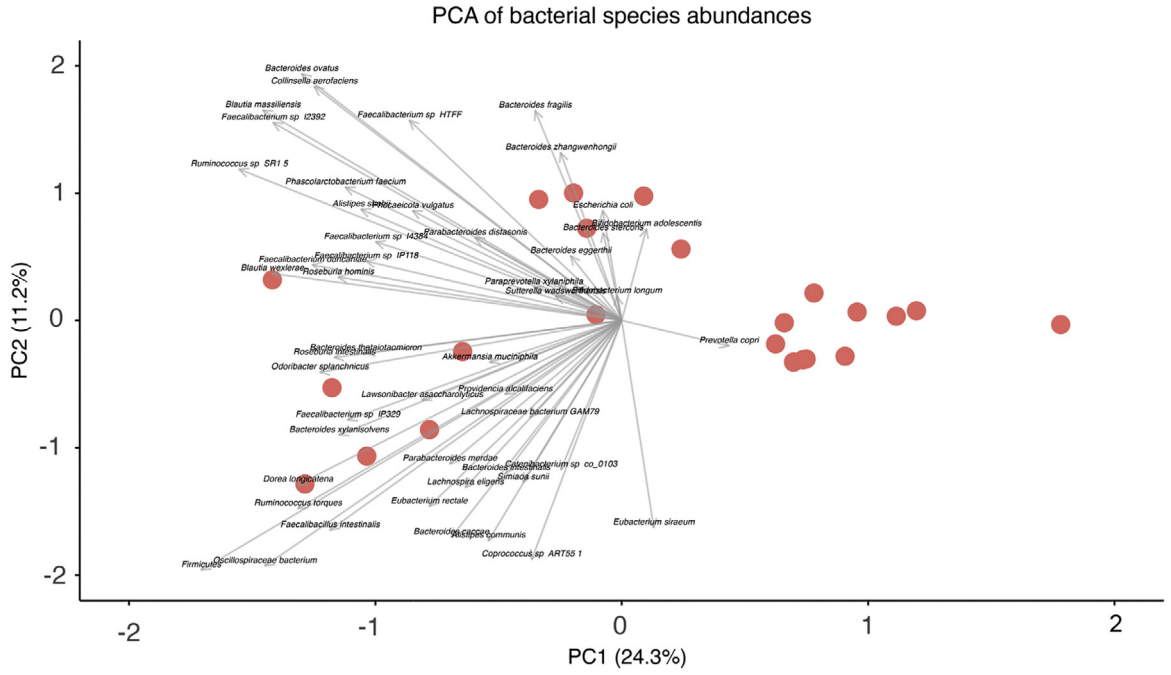

Fig. 4. PCA revealed that, of the identified bacterial species, *P. copri* was among the few components of PC1 (24.3%). *P. copri* = *Prevotella copri*; PC1 = first principal component; PC2 = second principal component; PCA = principal component analysis.

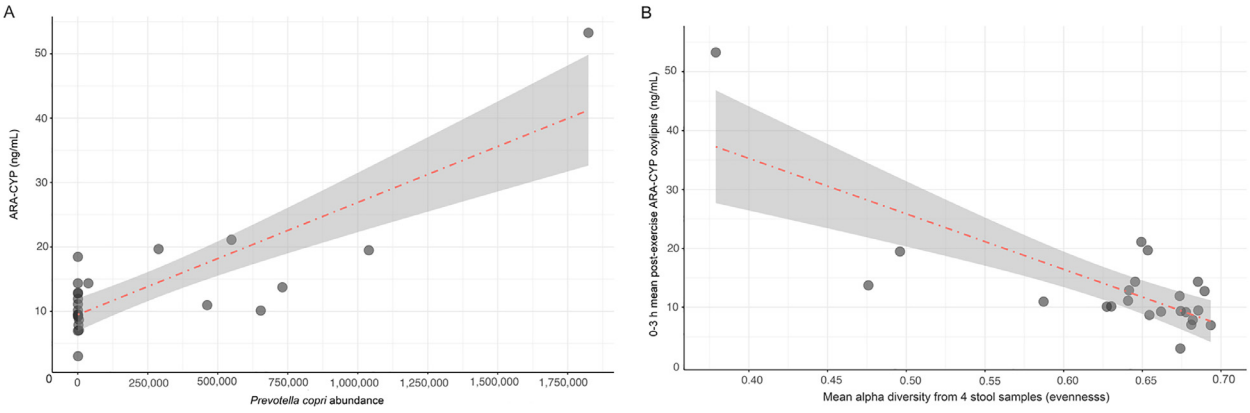

Fig. 5. (A) Linear regression between 0−3-h post-exercise oxylipin averages and average abundances of bacterial species identified just 1 significant relationship, and this was a positive correlation between *P. copri* and ARA-CYP ($R^2 = 0.676$, $p < 0.001$). (B) Negative relationship between post-exercise ARA-CYP and alpha diversity ($R^2 = 0.771$, $p < 0.001$). ARA-CYP = arachidonic acid-cytochrome P450; *P. copri* = *Prevotella copri*.

common microbe in the human gut of many adults, no clear consensus has emerged regarding its role in health.[17−25] Both favorable and unfavorable effects on human health have been reported, and health-related influences may depend on the metabolic context.[17,25] For example, *P. copri* has been linked to positive health effects including reduced visceral fat, improved glucose metabolism, enhanced bile acid metabolism and farnesoid X receptor (FXR) signaling, and quenching of superoxide radicals; but it has also been linked to insulin resistance, hypertension, persistent gut inflammation, and chronic inflammatory conditions.[17−25] *P. copri* has 4 distinct clades, each with substantial genetic diversity and differences in carbohydrate metabolism. These differences could explain the conflicting reports about *P. copri's* role in health.[25] *P. copri* contains enzymes that may degrade mucin and lead to increased intestinal permeability.[18] Also, together with a high-fiber diet, *P. copri* may enable the digestion of complex fiber and, as a result, lead to the overproduction of organic acids, including fumarate, succinate, and short-chain fatty acids, which can promote proinflammatory responses in macrophages.[24] On the other hand, recent murine data indicates that although *P. copri* increases intestinal barrier permeability, this may induce inflammatory tolerance and a related anti-inflammatory response in peripheral blood mononuclear cells.[26]

Although data are limited, a higher *Prevotella* abundance has been reported in athletes compared to sedentary controls.[27] *Prevotella* abundance has been linked to several factors important to athletic endeavor, including VO_{2max} and body mass index,[28,29] weekly average exercise duration,[30] and improved glucose metabolism.[31] In contrast, a linkage between

Prevotella and inflammation has been reported in athletes when in a resting state,[32] and the data from the current study indicate a strong relationship to elevated proinflammatory oxylipins after an acute bout of stressful exercise. Although habitual exercise training has been proposed to enhance human gut microbiome richness and evenness, over 50% of studies found no significant exercise effect.[33] The abundance of *Prevotella* in the gut is driven by multiple other factors, including host genetics, plant-rich dietary patterns, use of antibiotics, mode of delivery at birth, and method of infant feeding.[18,19] Thus, *Prevotella* abundance in the athletic population may vary widely due to these environmental, genetic, and lifestyle factors and be largely resistant to change during adulthood. Taken together, data from the few studies available suggest that athletic endeavor is positively related to *Prevotella* abundance but this may come at a metabolic cost (i.e., greater resting and post-exercise inflammation). On the other hand, *Prevotella*-related inflammation may induce training adaptations consistent with athletic endeavor.

The results from this study indicate that the oxylipins generated from the ARA fatty acid substrate and CYP-450 pathways were specifically related to *P. copri.* Underlying mechanisms explaining the linkage between *P. copri* and elevated post-exercise ARA-CYP remain to be explored. Growing evidence supports the influence that gut microbiome composition has on CYP expression in part through the release of gut-derived microbial metabolites.[34,35] Our research group reported that an acute bout of vigorous exercise augmented the release of gut-derived phenolic metabolites.[36] The specific role of *P. copri* in CYP expression is undetermined.

Strengths of this investigation included the analysis of 4 stool samples collected over a 10-week period using comprehensive WGS sequencing to establish gut microbiome composition and alpha diversity in 25 cyclists. Post-exercise inflammation was assessed using oxylipins involved in upstream regulation of inflammatory processes. Limitations included the lack of measurement of other inflammatory biomarkers known to be increased following sustained and vigorous exercise, including acute phase proteins such as CRP, complement C3, and serum amyloid A (SAA) as well as plasma cytokines such as IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), TNF-α, and IL-1 receptor antagonist. The time of day that stool samples were collected by the participants was not controlled. Yogurt and probiotic intake was not prohibited, but subjects were asked to maintain normal dietary intake patterns. The 4 stool samples collected from the subjects were tightly clustered, and analysis for beta diversity showed no differences in the microbiome profiles between the samples. This study did not determine the direct effect of *P. copri* on CYP expression. The relationship between *P. copri* abundance and pro-inflammatory oxylipins discovered in this analytical cross-sectional study needs to be examined further using higher level research designs, including an analysis of the *P. copri* genome, which could help explain the pro-inflammatory influence. This study was conducted in trained cyclists, and the findings may not apply to other groups. We did not explore the linkage of post-exercise inflammation with gut microbiome profiles collected from stool samples immediately after exercise. Thus, our findings should be applied within the context of gut microbiome profiles determined from stool samples collected the day before sustained and vigorous exercise. Alpha diversity was utilized in this study instead of other diversity indices because it is a broader measure encompassing both "richness" (the number of different bacterial species present) and "Shannon diversity" (a measure that considers both the number of species and their relative abundance).

5. Conclusion

This analysis showed a striking relationship between *P. copri* abundance in the gut microbiome and the extent of elevation in pro-inflammatory oxylipins during the 3-h period following 2.25 h of intensive cycling in 25 cyclists. Of 339 taxa identified as most abundant in the stool samples, only a single species, *P. copri*, was related, and this linkage was established for the most proinflammatory oxylipins generated from ARA and CYP. Ingesting carbohydrates and blueberries has been associated with reduced plasma levels of ARA-CYP oxylipins following intensive exercise and so is considered a beneficial practice for endurance athletes seeking to quell inflammation and enhance metabolic recovery. Thus, the association of high *P. copri* abundance with higher ARA-CYP inflammatory cytokines in about one-fourth of the study participants demands further exploration to determine the proper lifestyle and training recommendations for athletes and coaches. If our findings are confirmed by other groups, appropriate precision nutrition and exercise training guidelines could be established for the subgroup of endurance athletes identified as having high gut *P. copri* abundance.

Authors' contributions

DCN conceived and managed the study and drafted the manuscript; CAS and JCW recruited and scheduled the study participants, conducted the study, and helped write the manuscript; JL and KCL performed the statistical analysis and helped write the manuscript; AMO, FAM, and QZ participated in the oxylipin analysis and helped write the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary materials associated with this article can be found in the online version at doi:10.1016/j.jshs.2025.101039.

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