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Original research

Four men in a boat: Ultra-endurance exercise alters the gut microbiome

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ABSTRACT

Objectives: Compositional and functional adaptions occur in the gut microbiome in response to habitual physical activity. The response of the gut microbiome to sustained, intense exercise in previously active individuals, however, is unknown. This study aimed to prospectively explore the gut microbiome response of four well-trained male athletes to prolonged, high intensity trans-oceanic rowing, describing changes in microbial diversity, abundance and metabolic capacity.
Design: A prospective, repeated-measures, within-subject report.
Methods: Serial stool samples were obtained from four male athletes for metagenomic whole-genome shotgun sequencing to record microbial community structure and relevant functional gene profiles before, during and after a continuous, unsupported 33-day, 5000 km transoceanic rowing race. Calorific intake and macronutrient composition were recorded by validated food frequency questionnaire and anthropometry was determined by body composition analysis and cardiorespiratory testing.
Results: Microbial diversity increased throughout the ultra-endurance event. Variations in taxonomic composition included increased abundance of butyrate producing species and species associated with improved metabolic health, including improved insulin sensitivity. The functional potential of bacterial species involved in specific amino and fatty acid biosynthesis also increased. Many of the adaptions in microbial community structure and metaproteomics persisted at three months follow up.
Conclusions: These findings demonstrate that prolonged, intense exercise positively influences gut microbial diversity, increases the relative abundance of some bacterial species and up-regulates the metabolic potential of specific pathways expressing microbial gene products. These adaptions may play a compensatory role in controlling the physiological stress associated with sustained exertion as well as negating the deleterious consequences accompanying endurance exercise.

Practical implications

This study prospectively demonstrates that ultra-endurance exercise:

1. Increases gut microbial alpha diversity in healthy athletes.
2. Increases butyrate producing microbial species, species associated with improved metabolic health and insulin sensitivity.
3. Up-regulates functional metabolic pathways for microbial gene products including essential amino acids as well as some medium and long chain fatty acids.

1. Introduction

The gut microbiome plays a fundamental role in regulating host energy metabolism, oxidative stress, hydration status and systemic inflammatory responses.[1] Optimising these physiologi-

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cal processes and controlling any deleterious effects is essential to maintaining health and improving athletic performance. Interestingly, there is evidence to suggest that exercise exerts some influence over the gut microbial composition and function and it would appear that many of the compositional changes seen in gut microbial community structure associated with exercise are of benefit to the host.[2] The compositional changes associated with habitual physical activity include enhanced microbial diversity and increased abundance of beneficial microbial species.[3] Similarly, in high level athletes, studies have demonstrated compositional and functional variances in gut microbiota including increased microbial diversity in addition to enriched biosynthetic pathways for amino acid production, carbohydrate metabolism and short chain fatty acid synthesis.[3–5] To date however, it has proven difficult to prospectively study the effect of exercise on the gut microbiome of humans while successfully controlling confounding factors (such as diet, medications, inter-current illness etc.) Consequently, there is a paucity of prospective evidence to support the associations highlighted in existing observational studies.

This study aimed to explore the effect of prolonged, intense exercise on the gut microbiota community structure and activity in four well-trained male athletes who successfully completed an unsupported transatlantic row. These findings have implications for individuals regularly engaged in ultra-endurance exercise and may inform future research in athletes with high training volumes.

2. Materials and methods

This repeated measures analysis describes four healthy well-trained male athletes that completed a 4998.55 kilometres (km) (2699 nautical mile (nmi)) east–west transatlantic rowing race from La Gomera in the Canary Islands to Antigua in the Caribbean, in 33 days 22 h, averaging a distance of 151.8 km (82 nmi) per day. The crew of 4, split as 2 pairs, alternated every 2 h during the event, resulting in a cumulative rowing exposure for each individual of 394.9 h over the race period. Between each 2-h row interval, crewmembers had an opportunity to eat, sleep and attend to personal needs. Apart from adverse weather conditions and essential boat maintenance, interruptions to continuous rowing were kept to a minimum. The crew were completely unsupported throughout the race, finishing in sixth place in a field of 26 boats.

Ethical approval was obtained from the regional research ethics committee (Clinical Research Ethics Committee (CREC), University College Cork, Ireland) and the athletes were recruited to participate after they had declared their intent to participate in the transoceanic endurance event. All research was conducted in accordance with international standards for ethics in sport and exercise[6] and written consent was received from each participant prior to enrolment. Anthropometrics were measured pre and post-race on calibrated scales. An assessment of body composition was determined before and after the event using dual-energy X-ray absorptiometry (DXA). DXA measurements were made using a Lunar Prodigy Advance (GE-Healthcare) scanner.[7] A "7×4 min step" cardiorespiratory test protocol,[8] designed by the Australian Institute of Sport was performed on a rowing ergometer (Concept 2 Model) with respiratory gas analyser (Cosmed Quark CPET Metabolic Cart). This test was performed to establish detailed physiological information on the athletes' maximal performance parameters, submaximal capacity and efficiency. Peak respiratory exchange ratio (RER) (VCO_2/VO_2) during the VO_2 Max testing confirmed, that maximal effort had been achieved (RER > 1.15). Body composition and exercise physiology testing were repeated again two weeks following race completion. This two-week delay was designed to facilitate recovery whilst also negating any detraining effect associated with rest. Faecal samples were collected at four time points (pre-race, mid-way point (mid-Atlantic), immediately before race finish and at 3 months post race). The Omnigene® Gut microbial system was used to facilitate faecal collection and stabilisation of microbial DNA for gut microbiome profiling. Samples were collected and stored in accordance with manufactures standards and specifications. This collection method is used in circumstances where refrigeration and cold chain transportation is unavailable.[9] High throughput short-read (shotgun) sequencing was performed using the Illumina NextSeq 550 System platform®.[10] Microbiome analysis was conducted in the Teagasc Next Generation Sequencing Facility, in association with APC Microbiome Ireland.

Baseline dietary habits were assessed by way of a 146-item food frequency questionnaire (FFQ). This validated FFQ was an adapted version based on the original Willet FFQ.[11,12] Additional dietary information was available through the "My Fitness Pal" mobile application, including a detailed daily record of food consumed during the pre-race period. This was consistent with the FFQ recorded data, but not used for estimation of macronutrient data or energy intake. For a minimum of 6 weeks (maximum 6 months) before the race, all of the athletes had increased their calorific intake in anticipation of the increased calorific expenditure during the race. This is a common strategy in ultra-endurance athletes and is used to improve body energy stores in expectation of future high-energy demands. Intake was increased to approximately 21 megajoules (MJ) per day during this preparatory period. Average energy expenditure determined from a previous transoceanic rowing study was estimated to be 33.5 megajoules (MJ) per day.[13] The crew's eating strategy during the race was designed to match this anticipated energy expenditure. Food consumed during the race was recorded by a food consumption record. Daily food rations were prepared in advance of the race and each athlete consumed a similar ration. There was no fresh produce on-board during the race nor did they supplement their diet with fresh fish. Following the race, all athletes returned to their baseline diet.

Quality control processing of the sequenced stool sample data was performed by converting raw base call (BCL) files to FASTQ format, using bcl2fastq v2.19 software. FASTQ reads were then processed for downstream analysis through a quality control pipeline. Host (human) reads were removed using the NCBI Best Match Tagger (BMTagger version 1.1.0).[14] Reads were converted to Binary Alignment Map (BAM) format and sorted using FastqToSam version 2.7.1 software. Low quality reads with a Phred quality score less than 20 (Q < 20), adapter sequences and short reads with a length cut-off of 105 base pair (bp) were trimmed using trimBWAstyle.usingBam.pl script. Polymerase chain reaction (PCR) duplicates were removed using MarkDuplicates from Picard tools version 2.7.1. Finally, forward and reverse reads were merged and converted to FASTA format using IDBA fq2fa version 1.1.1.

Reads which passed quality-control filtering were used as input for taxonomic profiling using MetaPhlAn2.0[15] and Kaiju[16] version 1.5.0 utilising the non-redundant database in 'greedy' mode for additional sensitivity. The top 30 most abundant species were selected for visualisation using hclust2 python script (available here: https://bitbucket.org/nsegata/hclust2/downloads/). The phyloseq package (version 1.20.0) was used for alpha diversity analysis. Functional profiling of high-quality processed reads was facilitated by use of the Human Microbiome Project (HMP) Unified Metabolic Analysis Network (HUMAnN2 V.0.99) pipeline.[17] Models of microbial metabolic pathways produced by HUMAnN2 which measures UniRef cluster abundances by aligning reads against the ChocoPhlAn database were deployed. Metadata was associated with community totals using the HUManN2_associate package to identify altered pathways between samples. Spearman's False Discovery Rate (FDR) correction was used and a threshold of <0.05 was applied. Subsequent pathways were plotted using humann2_barplot package.

Table 1
Age, body composition and exercise physiology data, at pre-race, race finish and at two weeks following race completion presented as mean ± SD.

	Pre-race		Race-Finish[a]		Post-race (2 weeks)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	26.5	±1.3	–	–	–	–
Weight (kg)	82.6	±5.2	77.1	± 5.9	81.4	±5.9
Height (m)	1.8	±0.0	–	–	–	–
BMI (kg/m^2)	24.4	±1.4	–	–	24.1	±1.5
Body fat (%)	17.7	±7.9	–	–	16.2	±7.4
Lean body mass (%)	82.3	±7.9	–	–	83.8	±7.3
Resting heart rate (bpm)	70.3	±10.1	–	–	70.8	±7.9
Max heart rate (bpm)	195.8	±7.9	–	–	195.5	±6.8
VO_2 Max (l)	3.9	±0.4	–	–	3.8	±0.4
VO_2 Max (ml/kg/min)	48.16	±2.8	–	–	46.7	±1.5

[a] Race-Finish weights were recorded by the race organisers.

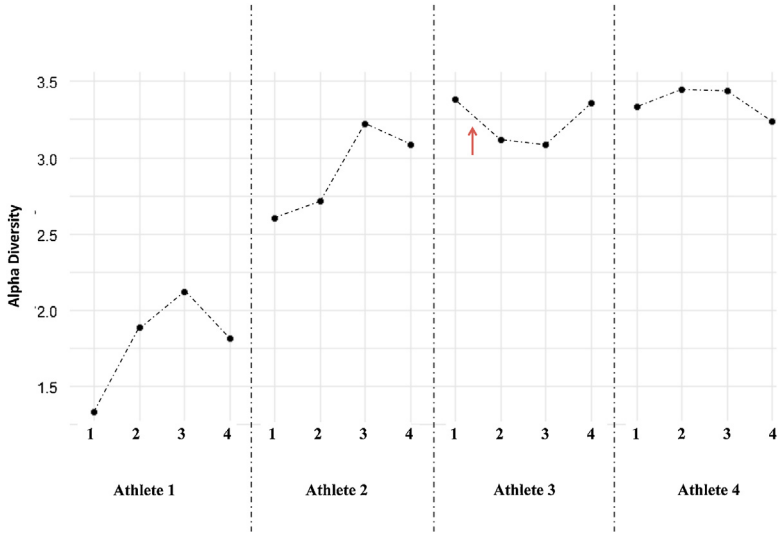

Fig. 1. Shannon Index of alpha diversity at all time points (pre-race, mid-race, race-finish and at 3 months follow-up) for each individual athlete. Numbers 1–4 indicate sampling time points, 1 (pre-race), 2 (mid-race), 3 (race-finish), 4 (3 months post-race). The red arrow indicates the time at which antibiotic consumption occurred in athlete 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

Group characteristics for the four male athletes including body composition and exercise physiology data, pre-race and at two weeks post transatlantic row are detailed in Table 1. Modest levels of aerobic fitness were recorded pre-race indicated by a mean VO_2 Max of 48.16 ml/kg/min. Aerobic capacity was maintained post-race with a mean VO_2 Max of 46.7 ml/kg/min recorded 2 weeks following race completion.

All 4 athletes increased energy intake to a target of 21 megajoules (MJ) for a minimum of 6 weeks pre-race. Intra-race calorie intake was similar at approximately 20.5 MJ per day. Pre-race macronutrient breakdown (based on food frequency questionnaire and standard serving size) consisted of fat (23.4%), carbohydrate (57.9%) and protein (18.7) while intra-race macronutrient breakdown (based on food consumption records) consisted of fat (17.9%), carbohydrate (67.15%) and protein (14.95%). FFQ derived pre-race estimates of non-digestible carbohydrate (fibre) intake was 21.45 g/day (SD ± 13.7), while intra-race fibre intake (derived from food consumption record/daily ration per athlete) was 23.1 g/day. While only subtle changes in macronutrient composition were identified between pre-race and intra-race diets, the diets themselves were constituently different. For practical reasons the majority of food on board consisted of rehydrated, freeze-dried, expedition type ration and non-perishable goods.

Changes in microbial, alpha (within-sample) diversity are displayed in Fig. 1. Shannon index is a measure that characterises species diversity within the microbiome community. Increases in alpha diversity were seen in all athletes apart from Athlete 3 who, notably, required treatment with oral antibiotics before the mid-race sample was collected (flucloxicillin 500 mg, q.d.s. orally for 7 days). Considering the three athletes that did not take an antibiotic during the race, increased diversity was seen as early as day 17 (mid-point) and continued until race end. A partial reversion was seen in two of these athletes and complete reversion to baseline diversity levels was seen in one at three months follow-up. The athlete exposed to oral antibiotic therapy had returned to baseline at three months post race (3.5 months post antibiotic exposure).

Fig. 2 details the variations in the 30 most abundant bacterial species, chronologically throughout the repeated sampling process using the MetaPhlAn2 metagenomic-profiling tool. Among these, *Dorea longicatena*, *Roseburia hominis* and unclassified members of the genus *Subdoligranulum* increased in all four athletes throughout the race while *Bacteroides finegoldii* reduced. In athlete 2, *Prevotella copri* increased in abundance from being below detectable levels to highly abundant at the last two time-points.

HUMAnN 2 software was used to functionally profile the presence, absence and relative abundance of metabolic gene pathways that showed a large increase in metabolic potential throughout the sampling process as well as the associated bacterial stratifications in which these changes were seen. Individual variation was evident, however there was a general pattern of increased abundance corresponding to metabolic pathways for certain essential amino

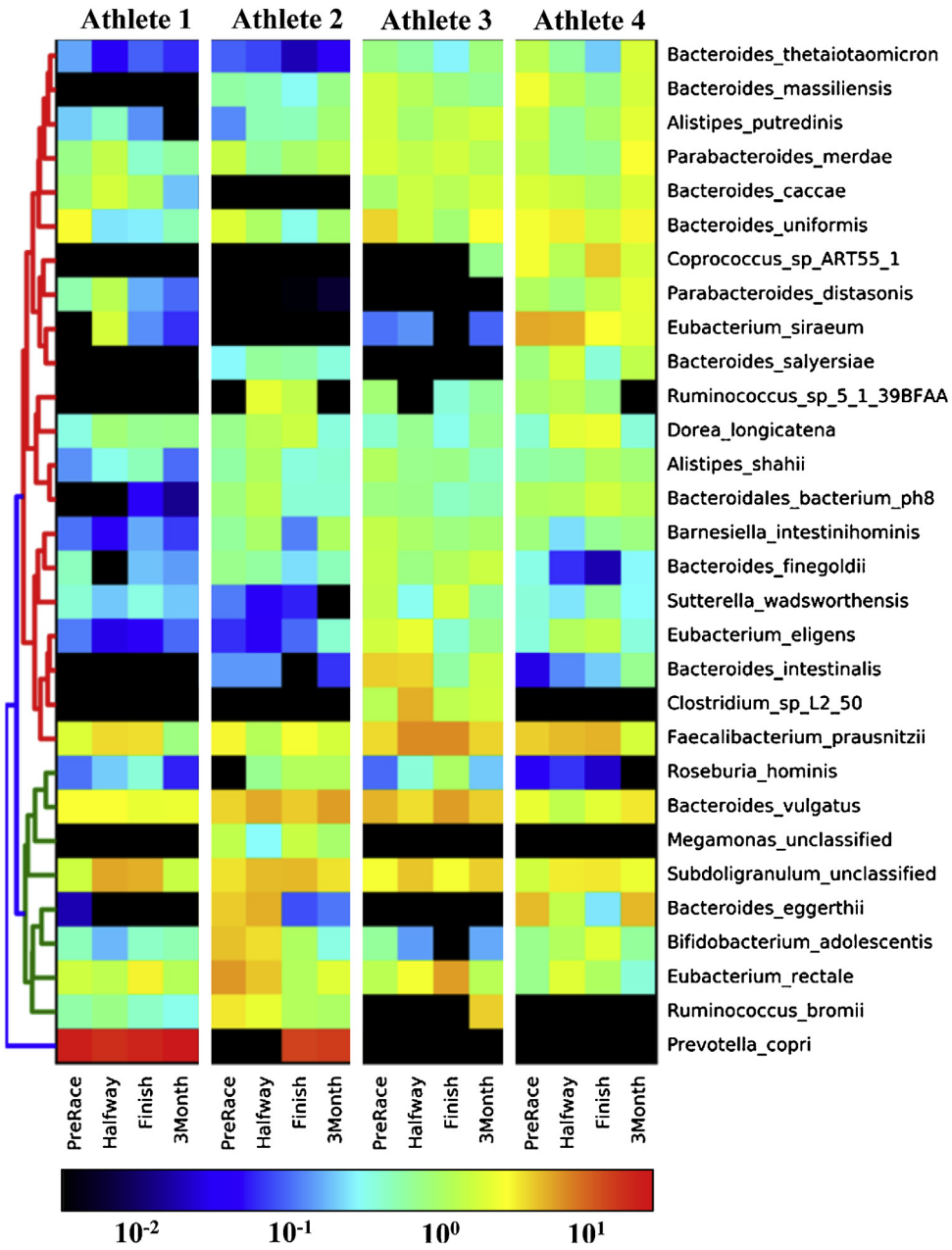

Fig. 2. MetaPhlAn2 metagenomic profiling of chronological changes in taxonomic composition of the 30 most abundant bacterial species for each individual athlete before, during, at finish and at three months following race completion. Branching on the left of diagram represents a phylogenetic tree of related bacterial species.

acids (L-isoleucine, L-lysine), some specific medium and long chain fatty acids (oleate, cis-vaccenate, palmitoleate and (5Z) dodec-5-enoate) as well as 5-adenosyl-L-methionine (SAMe) biosynthesis, fatty acid elongation and glycolysis. See Fig. S1.

4. Discussion

The gut microbiome is highly individualised in terms of the presence, absence and abundance of microbial species.[18] High diversity however, is one feature of the gut microbiome that is consistently cited as a metric of health. Previous studies have described an association between habitual exercise and increased alpha diversity, but this relationship has never been explored in the context of an exercise stimulus like human ultra-endurance. Additionally, how long it takes for exercise to induce these effects is unknown. In this study, a striking increase in alpha diversity was demonstrated in three of the four athletes. This change in diversity was evident as early as day 17 (race mid-point) and continued to increase until race end (day 33). This increase occurred independent of any significant change in cardiorespiratory fitness, with VO_2 Max similar pre- and post-race (Table 1). Two of the athletes continued to experience an increase in their alpha diversity at three months follow up, while the last individual had a correction to pre-race levels. The only athlete who experienced a reduction in alpha diversity had required oral antibiotics for cellulitis during the race. This collapse in microbial diversity was anticipated as antibiotics are known to exert deleterious effects on the gut bacterial community.[19] The athlete's diversity was subsequently found to have recovered to pre-race levels 3 months following race completion (Fig. 1). Antibiotic associated changes in taxonomic composition are well established, but research is also beginning to reveal more enduring effects of antibiotic exposure on the gut microbiome, including altered gene expression, protein activity and metabolism.[19] These negative consequences, supported by

our finding, should encourage deliberation before provision of antibiotic prescription in an athletic context.

Exercise induced changes in gut microbial composition are also known to be influenced by dietary extremes and this must be considered.[3] All athletes had increased dietary energy intake for a minimum of 6 weeks (maximum of 6 months) pre-race, with a target intake of 21 MJ per day consumed over that time period. Intra-race energy intake was analogous to the pre-race preparatory period at 20.5 MJ per day and is unlikely to have met the intra-race calorific demands of the athletes, which was estimated to be 33.5 MJ.[13] Macronutrient composition was also comparable before and during the race, however the variety and freshness of foods consumed during the race was reduced. Fibre (non-digestible carbohydrates) intake, which is known to favourably influence the gut microbiome, was also analogous before and during the race.[20] Controlling diet in studies of the relationship between the microbiome and exercise is challenging. While energy, macronutrients and fibre content were similar pre- and intra-race, the impact of freeze-dry food preparation used intra-race, particularly on the fibre content is uncertain.[21]

Of the bacterial species shown to increase during the race in all athletes (Fig. 2), *Roseburia hominis*, and members of the genus *Subdoligranulum* are known butyrate produces.[22] Butyrate, a short chain fatty acid (SCFA), plays an important role in regulating gut health, reducing inflammation and oxidative stress in addition to reinforcing the epithelial defence barrier and moderating visceral sensitivity.[22] A similar increase in SCFA production has been observed in other athlete studies.[4,5] *Dorea longicatena* also increased. This species has been associated with improving host metabolism and positively correlates with insulin sensitivity.[23]

In addition to an overall increase in alpha diversity and in the relative abundance of certain species, specific changes in community-wide metabolic gene potential also occurred. Up-regulation of genetic potential can result in the biosynthesis of specific, functional gene products. While these responses were variable between the athletes, there were some noteworthy trends observed. The relative abundance of genes involved in the biosynthesis of S-adenosyl methionine (SAMe) increased throughout the race and were maintained at 3 months follow-up. Bacterial species that demonstrated increased relative abundance of gene expression for the SAMe metabolic pathway included *Prevotella copri*, *B. vulgatus*, *F. prausnitzii*, *B. uniformis* and *E. rectale* (Supplementary file: Fig. 1, H). SAMe is involved in the metabolism of nucleic acids and polyamines, cell growth, survival and proliferation, and as a precursor of glutathione.[24] Glutathione has many effects including antioxidation, detoxification of xenobiotics and regulation of cell proliferation and immune response. It is perhaps glutathione's role as an antioxidant that is of most significance in this context given the high levels of oxidative stress induced by prolonged ultra-endurance exercise. Interestingly, a recent study on elite cyclists showed that a high abundance of the genus *Prevotella* was significantly correlated with time reported exercising during an average week.[5]

Gene expression of functional metabolic pathways involved in L-isoleucine and L-lysine essential amino acid production also increased. Bacterial species that demonstrated increased relative abundance of gene expression in L-isoleucine metabolic pathways included *coprococcus* sp. *ART55/1*, *R. intestinalis*, *P. freudenreichii* and in the case of L-lysine, *P. copri*, *F. prausnitzii*, *B. vulgatus* and *B. uniformis* (Supplementary file: Fig. 1, I, J). These essential amino acids play an important role in reducing muscular fatigue and damage to muscular integrity during strenuous exercise.[26] Additionally, studies have demonstrated that microbial derived lysine can contribute significantly to the body protein pool in humans.[25] Therefore, increasing this pathway may have anabolic effects. There is also evidence to suggest that changes in essential amino acid availability influences haematopoiesis, which in turn may increase oxygen carrying capacity and cardiorespiratory fitness.[26]

The relative abundance pathways involved in the biosynthesis of medium and long chain fatty acids also increased during the study (Supplementary file: Fig. 1, A–F). This included cis-vaccenate, oleate, palmitoleate and dodec-5-enoate biosynthesis as well as a pathway for fatty acid elongation. Fatty acids (medium and long chain) can be converted to energy through fatty acid oxidation, while some have more specific functions. *cis*-Vaccenate is an omega 7 fatty acid (long chain fatty acid) more typically derived from ruminant fat and dairy products,[27] while palmitoleate, another omega 7 fatty acid, is naturally found in animal fats as well as the oil of certain vegetables and nuts. Both of these fatty acids have roles in lipogenesis and catabolic energy metabolism as well as triglyceride, phospholipid and ester synthesis.[27] Additionally, some omega 7 fatty acids are known to be anti-inflammatory and to exert lipokine effects on autocrine and endocrine systems.[27] Likewise, oleate, an ester of oleic acid ((Omega 9) unsaturated fatty acid), has reported anti-inflammatory effects in addition to functions associated with improved metabolic health and immune function.[28] Whether up regulation of the metabolic potential of these fatty acids had a role in regulating inflammatory stress, improving catabolic energy production or anabolic creation of triglycerides and phospholipids is uncertain in this setting and requires further investigation, however any or all of these functions would be beneficial as an adaption to prolonged exercise and intense physiological stress.

5. Limitations

Controlling diet is the biggest challenge in investigating the relationship between exercise and the gut microbiome. While objectively, energy intake, macronutrient composition and fibre ingestion were similar throughout the pre-race and intra-race period, these diets were constituently different and consequently we cannot eliminate the potential confounding effects of this dietary change. This must be seen as a limitation. Secondly, while the within-subject study design is an effective method of demonstrating the highly individualised nature of the gut microbiome as well as the relevance of omic technology in a sporting context, the small sample size must be seen as a limitation. Larger prospective studies are necessary to explore these findings. Finally, this study supports the findings of prospective murine models and observational human studies that suggest exercise favourably influences gut microbial community structure and function. However, when interpreting these finding it is important to recognize that the findings in this study occurred in the context of a sustained, ultra-endurance exercise stimulus beyond which most athletes are routinely exposed.

6. Conclusion

This study is the first to demonstrate that ultra-endurance exercise increases gut microbial diversity, some butyrate producing species and up-regulates the metabolic potential for specific functional gene products in well-trained athletes. These changes were determined prospectively and in the context of a diet that was constituently different but comparable in terms of energy, macronutrient composition and fibre throughout. Additionally, the changes in microbial community structure and function occurred in the absence of any significant improvements in cardiorespiratory fitness. These findings improve our understanding of how the gut microbiome responds to sustained aerobic exercise and may have implications for athletes regularly competing in ultra-endurance events as well as individuals with high training volumes. Larger studies are needed to explore these associations in more detail.

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Contributors

DK, MM, FS, POC, SU and OC were responsible for study concept and design. TW and DK conducted the exercise physiology testing. RW, OOS, PC performed the microbiome analysis and biostatistics. All authors (DK, MM, FS, OC, POC, SU, TW, RW, OOS, and PC) contributed to the written manuscript.

Patient consent

Written informed consent was received from each participant prior to enrolment.

Ethical approval

Sanctioned by the Clinical Research Ethics Committee (C.R.E.C), University College Cork, Lancaster Hall, 6 Little Hanover Street, Cork, Ireland. All procedures performed were in accordance with the ethical standards of the aforementioned institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jsams.2019.04.004.

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