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The Gut Microbiota Characterization of a World-Class Mountain Trail Runner During a Complete Competition Season: A Case Report

Jesus Álvarez-Herms, PhD, ATC*†; Martin Burtscher‡; Adriana González-Benito, MS*; Francisco Corbi, Sport Science§; Adrian Odriozola-Martínez, PhD*

*Hologenomiks Research Group, Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country, Spain; †Phymolab, Physiology and Molecular Laboratory, Segovia, Spain; ‡Department of Sport Science, University of Innsbruck, Austria; §Institute of Physical Education of Catalonia (INEFC), Faculty of Sport Science, Lleida, Spain

In the present case study, the gut microbiota (GM) profile of a male elite mountain runner (34 years, 171 cm, 59 kg, VO_{2max} = 92 mL/min/kg) was analyzed over a 5-month competitive period (6 samples). Gut microbiota diversity increased throughout the season, where higher levels coincided with peak performance, and shorter and longer races (42 km versus 172 km) produced different phenotypic GM changes. Shorter races promoted elevation of protective bacteria related to positive benefits (higher production of short-chain fatty acids, lactate resynthesis, and mucin degraders). By contrast, longer races promoted an elevation of opportunistic pathogenic bacteria while reducing protective commensal bacteria. The present findings indicate that a higher resilience of the GM after competitions may support rapid recovery from maximal exercise. Gut microbiota analyses before and after competition could represent a rapid indicator for the (patho) physiological impact of exercise and provide information on gut health and the recovery time needed.

Key Words: gut microbiota, endurance exercise, physiological profile, training load

Key Points

- Physical fitness is directly related to systemic health, likely including the gut microbiota, which might contribute to reduced gastrointestinal distress during ultraendurance events.
- Characterization of the gut microbiota in athletes might support the assessment of different physiological states related to metabolism, immunomodulation, and/or anti-inflammatory resilience capacity.
- Longer duration of physical exercise produces higher impacts on gut microbiota homeostasis than shorter-duration exercise at maximal intensity.

The gut microbiota (GM) is a community of bacteria, fungi, viruses, and other microbes that colonize the digestive tract, interacting with each other and with the host.[1] The National Institutes of Health Common Fund has been supporting the investigation of human microbiome projects since 2008, with the mission of generating resources that would promote the understanding of how the microbiome impacts human health and disease.[2] Meanwhile, thousands of studies have collected samples from healthy people and/or patients to expand the metadata and link bacteria and the GM ecosystem with specific phenotypic gut/systemic functions and diseases.[3] The relationship between the host and the GM involves an intricate symbiosis that helps maintain gut health and other physiological functions related to digestion, immune modulation, energy efficiency, systemic acidosis recycling, pH, and detoxification.[4] Previous studies have demonstrated how a higher abundance and diversity of microorganisms in the gut can contribute to the proliferation of species responsible for the improvement of gut functions, including intestinal permeability (mucus layer) and/or the production of short-chain fatty acids (SCFAs).[5] The symbiotic model describes how the microbiota performs important functions for the host, protecting from infections and pathogenic colonization, contributing to nutrient metabolism, and training gut immunomodulation; on the other hand, the host provides nutrient-rich niches and environmental conditions to ensure the survival of its resident bacterial communities.[6]

During the last 2 decades, analyses of fecal samples have led to the description of at least 1000 different bacterial species, which have been divided into specific groups with protective or pathogenic relevance.[7] Furthermore, different stimuli (eg, nutrition; exercise; chrono regulation; mode of birth; stress; and exposure to medication, toxins, or pollution) seem to influence the composition of individual GM.[7]

Once GM homeostasis is compromised by the growth of certain species with pathogenic potential, this can elicit adverse

effects on the gut and systemic health (eg, systemic endotoxemia, immune overactivation and inflammation, and gastrointestinal distress), likely associated with impaired physical performance.[8] The change in microbial composition promoting an imbalance between beneficial and potentially pathogenic bacteria is termed "dysbiosis."[9] Dysbiosis, in turn, leads to a lower microbial diversity with reduced metabolic activity and a loss of the symbiotic relationship between the host and its resident microorganisms.[10]

Ultraendurance athletes need to ingest sufficient energy during exercise to maintain physical performance. However, in the presence of intestinal dysbiosis, gastrointestinal problems during exercise may occur, accompanied by a reduced ability to ingest nutritional energy without discomfort (for example, diarrhea, reflux, cramps, nausea, etc).[11,12] Consequently, maintaining a healthy GM balance will reduce gastrointestinal discomfort during exercise and increase the likelihood of appropriate energy intake.[13]

Composition of the GM differs among individuals and depends on multiple factors mainly related to lifestyle. Nutrition is key to promoting higher proportions of fermentative bacteria, which are capable of producing SCFAs from complex carbohydrates and positively benefit gut health and physical performance in athletes.[14,15] In this regard, Genera such as *Bifidobacterium*[14] and *Lactobacillus*[16] are considered protective commensal bacteria because they were found to be elevated in healthy people and athletes with better performance.[14] Other organisms such as *Akkermansia muciniphila*, *Faecalibacterium*, and *Roseburia* have been suggested to be important modulators of mucolytic activity related to healthy intestinal permeability.[7] Conversely, daily routines that alter circadian rhythm and promote chrono disruption,[17] Western-style diets,[18] or the use of antibiotics[19] can negatively affect GM homeostasis and induce gut dysbiosis with negative impacts on physical health conditions.[9] The specificity of nutrition based on certain macronutrients is an important modulator of the GM. Athletes following a diet consisting of rich sources of animal proteins and simple carbohydrates might be associated with a *Bacteroides* GM enterotype with more pathogenic potential than other enterotypes such as *Prevotella* (diets rich in complex carbohydrates).[20,21] Currently, the specific nutritional paradigm is based on the importance of constantly maintaining energy flux during exercise through increased consumption of rapid carbohydrates.[22] Moreover, lower intake of fiber and beneficial lipids can have detrimental effects on GM homeostasis and health.[23]

The physiological strain and metabolic demands of ultraendurance races are extremely high.[24,25] So far, 2 recent studies reported that ultraendurance competitions can acutely modify the GM.[26] The first study conducted by Grosicki et al examined changes in the GM in a world-class ultramarathon runner before (21 and 2 weeks) and after (2 hours and 10 days) a 163-km competition.[26] They observed that, immediately after finishing the competition, α-diversity (Shannon Diversity Index) and phylum-level bacterial composition increased. The abundance of other bacteria also changed after the race but returned to basal levels 10 days later. The abundance of some pathogenic bacteria, such as *Streptococcus* or *Haemophilus*, increased considerably. The second study by Sato et al analyzed the GM before and after (10 days) a 96-km mountain race.[25] This study also found that the Shannon Diversity Index increased significantly in some individuals but not collectively. Of relevance, a decrease in the abundance of butyrate-producing bacteria was observed after the race, which is compatible with affecting immune function at the intestinal level.[25] In summary, both studies hypothesized that a brief modification of the GM may provoke an immunosuppressive window that favors illnesses and infections.

The GM is a dynamic structure that rapidly responds to endurance exercise; however, previous reports have not documented how these acute and chronic changes are evolving during a complete season, for example, in ultraendurance athletes. Obtaining appropriate biological samples from elite athletes is difficult but essential for the characterization of specific phenotypes related to such physiological demands. Here, we had the unique opportunity to characterize the GM profile in 1 very elite mountain runner during a complete season. It has been suggested that a healthy GM ecosystem could provide benefits for endurance and ultraendurance athletes by reducing and protecting against gastrointestinal discomfort and complaints during competitions. This protection would be the result of faster recovery after long and intense exercise due to diminished systemic inflammation, increased immune suppression, and increased hepatic gluconeogenesis from recycling acidosis and other residual metabolites produced during exercise.[5,25,26] Thus, considering the symbiotic model between host and GM, characterization of the "athletic model" for excellence may contribute to the understanding of the most favorable GM phenotypes in both athletes and the normal population.

This study includes data on training load and GM recorded during a complete season and after very popular races, that is, Zegama-Aizkorri (ZGM) and the Ultra-Trail of Mont-Blanc (UTMB).

The Participant

A professional male mountain runner (34 years, 171 cm, 59 kg, peak VO_{2max} = 92 mL/min/kg) was physically monitored over a 5-month period (2022), including during 4 popular races (ZGM, Hardrock 100 miles, Sierre Zinal, and UTMB). Besides training loads and race performance, 6 GM samples were obtained and analyzed, including those before and after the ZGM and UTMB races. The characteristics of the races are available at https://www.zegama-aizkorri.com/ and https://montblanc.utmb.world/es/races/utmb.

The athlete followed a consistent diet during the complete season, focusing on high intake of carbohydrates (from vegetables and fruits of complex origin) and low intake of animal protein sources. The proportions of macronutrients during the season varied from 54% to 63% for carbohydrates, from 17% to 23% for fats, and from 20% to 22% for proteins. The athlete consumed, on average, 4.1 L of fluid per day, and the daily mean energy intake from food consumption was 4700 kcal. During the year, dietary supplements consisted of specific vitamins, such as vitamin D (during the winter) and vitamin B12 (during the overall year), and fatty acids, such as omega 3. The athlete did not take any medication regularly. The VO_{2max} test was performed at Catalonia General Hospital (Sports Medicine unit) with a Harbor-UCLA Bike applying a 25-W ramp protocol.

Fluid intake during the races ranged from only 0.75 L during the ZGM race to 6 to 7 L during the UTMB race. The total energy and proportions of macronutrients ingested during the races differed considerably. During the ZGM race, the intake only consisted of simple CH from commercial sports products (gels and drinks with carbohydrates), approximately

Table. Performance Characteristics of 4 Competitions: Zegama-Aizkorri (ZGM), Sierre-Zinal (SZ), Ultra Trail of Mont-Blancblanc-Chamonix (UTMB), and Hardrock 100 Miles (HR100)

Race	Time, (h:min:s)	Time (h:min:s) Spent in Various Heart Rate Zones (bpm)					Overall Ascent (m)	Pace (min/km)	Cadence (steps/min)	Average Heart Rate (bpm)	Lactate Post (mmol/L)
		<120	120–145	146–165	166–184	>184					
ZGM	3:36:02	0:01:00	10:46	1:52:08	1:32:01	0	2557	5.09	171	164	8.8
SZ	2:30:15	0:01:00	0:03:27	0:34:20	1:46:55	4:36	2118	4.48	172	171	—
UTMB	19:50:00	3:23:30	10:34:55	5:04:26	15:17	0	9615	6.52	155	135	13.3
HR100	21:36:30	2:30:16	13:25:15	4:51:10	8:32	0	9380	8.01	145	135	—

Abbreviations: bpm, beats per minute; HR100, Hardrock 100 miles; SZ, Sierre-Zinal; Ultra Trail of Montblanc-Chamonix; ZGM, Zegama-Aizkorri.

700 kcal with 3500 kcal expended, whereas the intake during the UTMB race was approximately 9000 kcal consisting of 81% CHO, 4% proteins, and 15% fats, with a total energy expended of 14 500 kcal.

Lactate levels measured (Lactate Scout+, EKF Diagnostics) immediately after each race (between 8 and 12 minutes) differed between 8.8 and 13.3 mmol/L in the ZGM and UTMB, respectively (Table).

GM Assessment

The athlete was adequately informed about the risks and benefits associated with study participation, and the present study was approved by the Human Research Ethics Committee of the School of Science and Technology (M10 2021 191). The GM of the athlete was monitored by sample collection of feces between 7:00 a.m. and 8:00 a.m. before and after each competition using the DANASTOOL Sample Collection Microbiome kit from DANAGEN following the manufacturer's instructions. The methods used for analysis are available in the Appendix.

COMPARATIVE OUTCOMES

Physiological Performance During the Training Season and Competitions

The approximate accumulated training volume of the athlete during the season is shown in Figure 1. The physiological strain (indicated by heart rate zones; Table) of the competitions was different when comparing shorter and longer trails. In shorter races (ZGM and Sierre Zinal), running intensities were very high, as indicated by the recorded average heart rate (92% of predicted maximal heart rate) and speed. However, even though >70% of the race was performed at lower intensities in the longer races (UTMB and Hardrock 100 miles), the total time accumulated at moderate intensities (146 to 166 ppm) was longer than in the ZGM race (Table). Neuromotor patterns related to stride, pace, cadence (stride frequency), and ascent speed were also different between short and long races (Table). Lactate levels measured immediately after the races differed between 8.8 and 13.3 mmol/L in the ZGM and UTMB, respectively (Table).

GM Analysis

Analysis of the GM revealed an increase in α-diversity throughout the season (5.09 to 7.90; Figure 1). However, after the UTMB, the diversity of the GM increased, possibly related to a relevant growth of pathogenic species (Figure 2). Moreover, during the season, and particularly after races, we observed modifications of specific levels of microorganism (Figure 2). The short race (ZGM) promoted fewer increases in bacterial species with pathogenic potential than longer races (UTMB), and different commensal protective species were found to be increased (Figure 2). Of interest, the shorter

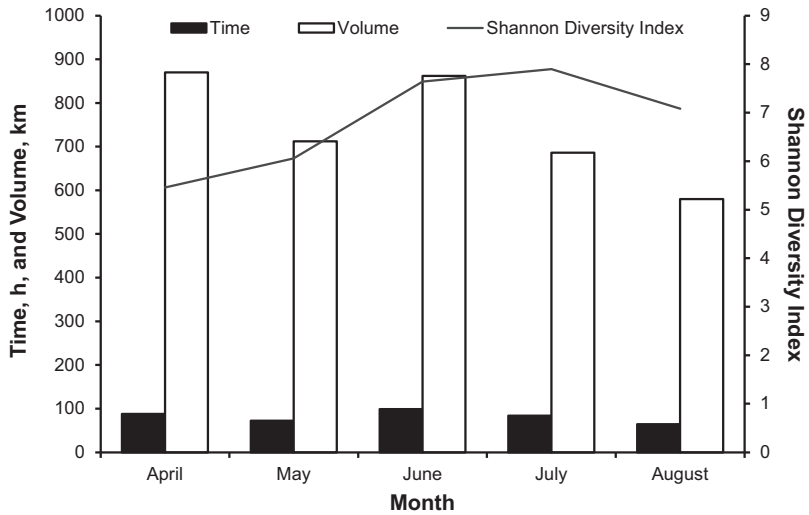

Figure 1. Training load during the competitive season and Shannon Index (gray line).

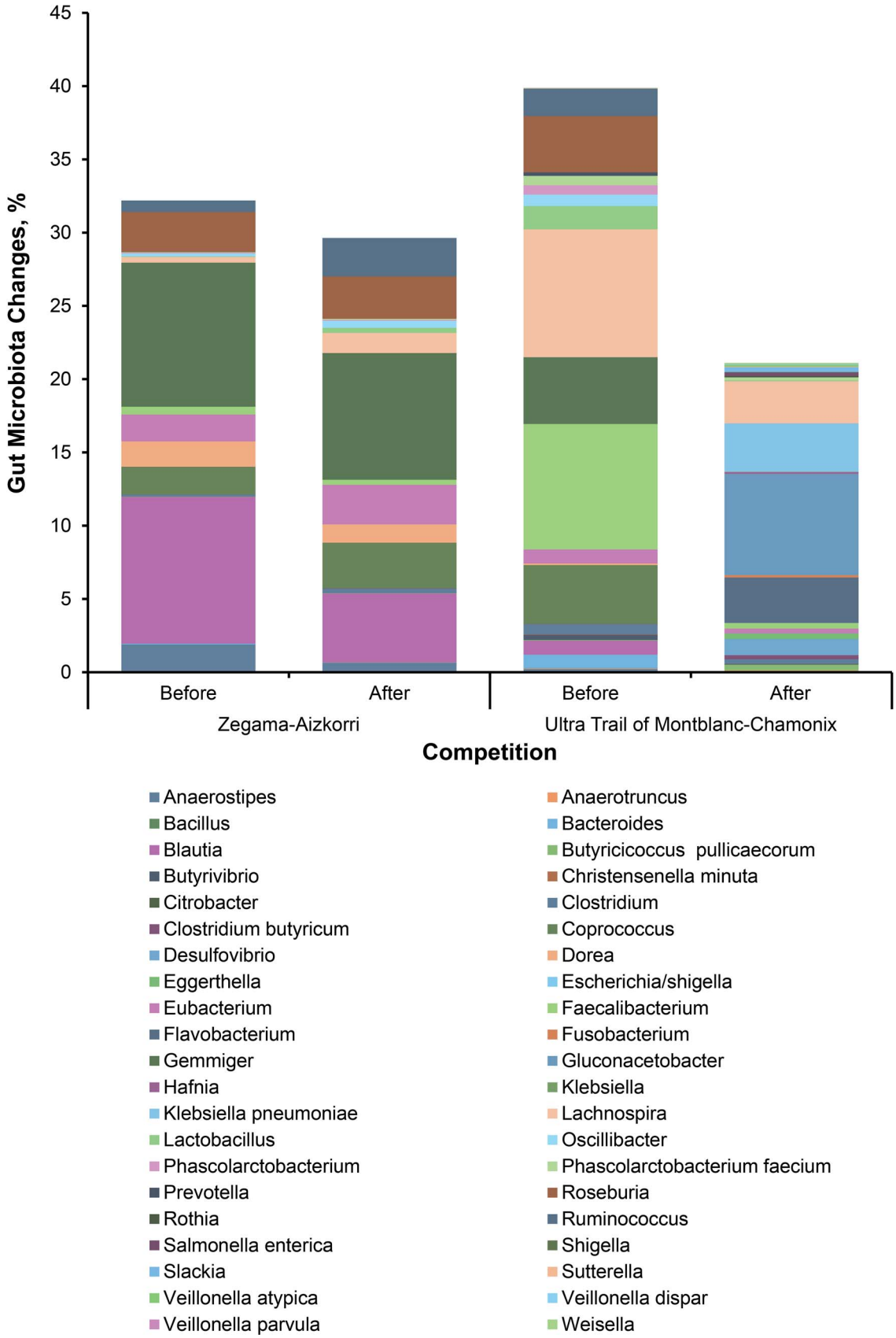

Figure 2. Changes in gut microbiota bacteria after the Zegama-Aizkorri and Ultra Trail of Mont-Blanc races. Colors represent the proportions of species in each sample (before and after). Colors indicate the relative proportions of the total species in the sample.

race (ZGM) did not promote any increases in pathogenic species as was observed after the longer race (UTMB).

DISCUSSION

This study provides a novel and unique characterization of the GM in a legendary mountain athlete that could be of practical/clinical interest to understanding changes in the GM during a competitive season, including measurements obtained before and directly after extreme endurance competitions. The main results of the present case study indicate that (1) increases in volume and intensity of endurance training through the season promoted a higher GM α-diversity (Shannon Diversity Index) and stimulated a different specialization of microorganism proportions after races; (2) GM findings after the races showed changes in proportions of specific bacteria, where ZGM (the shorter race) stimulated pronounced increases in species favoring positive actions on gut homeostasis and health and the UTMB (the longer race) promoted a transient GM dysbiosis with proportional increases in pathogenic species; and (3) the athlete showed a high resilience and stability of protective GM species. The athlete did not report any gastrointestinal inconvenience during or after the races and did not report any musculoskeletal injury during the season.

The taxonomic richness of the GM is indicative of gut health, higher versatility of metabolism, phenotypic resilience, and improved physical performance.[3] In the present athlete, the α-diversity index (described by the Shannon Diversity Index) was found to be significantly higher than previously reported in other trail runners (±2.7 and 3.6 versus 7.9 here).[25,26] In agreement with Fernandez-Sanjurjo et al, higher versatility of the GM could be a determinant for better endurance performance, as we confirmed.[14] Fluctuations occurred in response to certain training events (eg, injury, illness, and training peaks), as was also reported by Karl et al in association with the growth of potentially harmful bacteria, as was observed after the UTMB.[27]

The GM profile in the present athlete indicates an important proportion of bacteria capable of producing SCFAs (specially butyrate) to promote mucolytic activity and regulate anti-inflammatory and metabolic processes in the gut. In this regard, after the ZGM, relevant increases were found in some commensal bacteria (species) considered to be protective and healthy, such as *Anaerotruncus*,[28] *Butyricoccus*,[29] *Butyrivibrio*, *Clostridium butyricum*,[30] *Eubacterium*,[31] *Roseburia*,[32] *Romboutsia*,[33] *Ruminococcus*,[33] *Lactobacillus*,[16] *Lachnospira*,[34] *Oscillibacter*,[35] *Phascolarctobacterium*,[36] and *Coprococcus*.[37] In general, these bacteria have been suggested to be beneficial for mucolytic activity, promoting homeostatic gut health and improving effective intestinal permeability, the production of SCFAs (specially butyrate), favorable anti-inflammatory responses, and effective pathways to metabolize lactate in the gut from bloodstream sources.[5] Moreover, *Lachnospira* and *Coprococcus* increased remarkably only after the ZGM, in agreement with previous studies where such changes were associated with better physical fitness.[38] The postrace elevation of some bacteria related to the production of butyrate (*Anaerotruncus*,[28] *Butyricicoccus*,[29] and *Clostridium butyricum*[30]) could be indicative of protective mechanisms of gut homeostasis and systemic metabolism.[7] Another bacterium related to intestinal permeability and gut health is *Ruminococcus*, which was found to be increased after the ZGM and reduced after the UTMB. In this regard, Craven et al had reported increased *Ruminococcus* through the season in athletes who were accumulating higher training volumes.[39]

Elevated prerace abundances of *Roseburia* and *Faecalibacterium* may be indicative of protective mucolytic activity and may also be related to muscle anabolism, possibly contributing to fast recovery.[40] However, similar to the study by Karl et al, postexercise measurements showed a decrease of *Faecalibacterium* after both the ZGM and UTMB and *Roseburia* after the UTMB.[27] Another bacterium that changed in abundance after the races was *Oscillibacter* (increased after the ZGM but decreased after the UTMB), which might have contributed to the accumulation of uremic metabolites through pyruvate metabolism associated with serum lactate levels.[41] The activity of *Oscillibacter* likely also promoted changes in intestinal pH after the UTMB. A protective GM ecosystem can be favorable to promoting a niche for the intestinal lumen and preventing colonization of pathogens sensitive to changes in acidic pH.[4] Thus, our findings may indicate (Table) that large time accumulated above the anaerobic threshold (as during the UTMB) with a transient change of acid-base homeostasis could promote a reduction in SCFA production as an indirect consequence of changes in luminal pH, favoring the opportunistic growth of pathogens.[42] This might partially explain why (very) long distance running acutely modifies the internal gut milieu (pH) and promotes the rapid growth of certain pathogenic bacteria. In fact, comparing the pre-post UTMB versus ZGM races, the abundances of SCFA-producing bacteria such as *Butyrivibrio*, *Coprococcus*, *Eubacterium*, *Anaerostipes*, *Blautia*, *Faecalibacterium*, *Lachnospira*, and *Gemmiger* decreased. Consequently, the UTMB promoted a transient dysbiosis of the GM compared with the ZGM, with a proportional increase in pathogenic species as *Klebsiella*, *Citrobacter*, *Fusobacterium*, *Gluconacetobacter*, *Salmonella enterica*, *Shigella*, and/or *Kluyvera*, that were not found after the ZGM.

Findings from the present study should be interpreted in the context of a unique case report. Therefore, the extrapolation of the present results is limited to this unique athlete. Previous studies analyzing GM phenotypes have indicated that protective bacteria may contribute to the explanation of physiological performance and recovery. However, these explanations remain speculative due to the inability to concisely monitor variations in diet and other factors that may alter the GM (eg, training at cold or hot ambient temperatures). Highlights include that the present study is the first to characterize acute and long changes in gut microbial ecology in 1 extremely successful mountain runner during a complete competition season.

The different trends on bacteria growth after short versus long races indicated that physiological demands and systemic stress promote rapid changes in the GM possibly related to the specific diet associated with the metabolic demands and other factors related to exercise intensity and lactate concentration, hypoxia, and thermal stress during and following races. However, the different analyses from the GM samples during a 5-month period showed a clear stability and resilience on bacterial proportions. In agreement with Grosicki et al, the robust stability of the GM, even during postmaximal ultraendurance exercise, could be related to high-fit performance.[13]

Clinical Bottom Line

The presented case study indicates that exceptional aerobic fitness can be associated with a higher GM diversity and

elevation of bacteria related to SCFA production. Moreover, GM diversity increased in parallel to peak performance and higher volume and intensity of exercise. Short, intense competitions stimulated the elevation of protective commensal bacteria associated with beneficial effects on gut barrier permeability and SCFA production. By contrast, UTMB promoted a higher elevation of pathogenic bacteria. Rapid recovery of the basal GM profile after races indicates a high resilience of the GM, contributing to the maintenance of gut health and the prevention of gastrointestinal complaints during races.

Gut microbiota analyses in endurance athletes could be a promising tool to monitor gut health and provide rapid information over adaptive phenotypes related to exercise and microbial resilience. Considering the individuality of the GM, further research will be performed, including repeated analysis in different phases of training, competitions, and recovery states to characterize the GM of athletes and their resilience. Moreover, evaluating modulatory effects of the factors influencing the GM is key for both athletes and the nonathletic population. Furthermore, comparing GM characteristics of individuals performing different types (eg, high- and low-energy consuming) of sport will help to create a more complete picture of potential interactions between GM and exercise performance.

This case report indicates that individual GM analyses over a competitive season in a top athlete can provide valuable information about the interaction between GM, psychophysiological stress, diet, and performance. Prerequisites for the successful inclusion of GM analyses in training control are the use of appropriate methods of analyses and proficient interpretation of results. This means a close working cooperation between athletes, coaches, scientists and supervising doctors, therapists, and nutritionists. Existing study findings, including case reports from elite athletes (like this one), may form a helpful basis for the application and interpretation of such analyses. However, more data and well-designed studies are needed before GM analysis can be introduced into general training practice.

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Address correspondence to Jesus Álvarez-Herms, PhD, ATC, Hologenomiks Research Group, Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country UPV/EHU, Leioa 48080, Spain. Address email to jesusah80@gmail.com.

Appendix. Laboratory Procedures to Analyze Gut Microbiota (GM) Samples

Gut Microbiota Analysis

Samples were stored after collection at −80°C until DNA extraction. Other samples taken during the season were obtained in the same way. DNA was extracted using the DANAGENE Microbiome Fecal DNA kit. DNA quantitation was performed by fluorometry (Qubit 2.0, Life Technologies, Thermo Fisher Scientific) using an HS dsDNA Assay (Thermo Fisher Scientific) and spectrophotometry (NanoDrop 2000c, Thermo Fisher Scientific). PCR amplification of full-length 16S rRNA genes (1500 pb) was conducted using a 16S Barcoding kit (SQK-RAB204, Oxford Nanopore Technologies) containing the 27F/1492R primer set and LongAmp Taq 2× Master Mix (New England Biolabs). Amplification was performed using an Applied Biosystems Veriti thermal cycler (Thermo Fischer Scientific) with the following PCR conditions indicated by the manufacturer: initial denaturation at 95°C for 3 min, 25 cycles of 95°C for 20 seconds, 55°C for 30 seconds, and 65°C for 2 min, followed by a final extension at 65°C for 5 min. PCR products were purified using Clean NGS (CleanNA, PH Waddinxveen) and quantified by fluorometry (Qubit 4.0, Life Technologies, Thermo Fisher Scientific) using an HS dsDNA Assay (Thermo Fisher Scientific). A total of 100 ng of DNA was used for library preparation, and MinION sequencing was performed using a MinION Nanopore DNA sequencer, ADP-FLG001 Flonge adapter, and FLO-FLG001 (Oxford Nanopore Technologies) according to the manufacturer's instructions. minKNOW UI software version 21.02.01 (Oxford Nanopore Technologies) was used for data acquisition and base-calling converting sequence reads (ie, FAST5 data) into FASTQ files using the Guppy version 3.2.10 pipeline (Oxford Nanopore Technologies). The barcoding workflow in the Metrichor Ltd analysis platform EPI2ME (Oxford Nanopore Technologies) was used to identify bacteria at the genus and species levels. For that purpose, FASTQ files were uploaded to the EPI2ME desktop agent 16S workflow (Oxford Nanopore Technologies), where real-time classification was performed using the NCBI 16S rRNA gene BLAST database. The BLAST was run using the parameters max_target seqs = 3 (finds the top 3 statistically significant hits). All read classifications were filtered for >77% accuracy and >30% coverage, removing non-specific alignments. Obtained results were downloaded as a comma-separated values file. In-house software results were processed to avoid infra-representation of each taxonomical ID and convert reads in relative abundance according to the estimated 16S copy number for each bacterium based on the rrnDN database. ZymoBIOMICS gut Microbiome Standard (Zymo Research) was used as control DNA and analyzed twice following the same lab methodology of the case study to determine the sensitivity and specificity of the molecular analysis. A-Diversity was used to describe the microbial diversity of an ecological community. It was calculated using mathematical measures after the GM analysis for species richness.