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Compositional and functional profiling of the gut microbiota in middle- and long-distance runners: a longitudinal study with personalized nutritional intervention

Profilo composizionale e funzionale del microbiota intestinale in mezzofondisti e fondisti: studio longitudinale con intervento nutrizionale personalizzato

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Abstract

The gut microbiota is increasingly recognized as a key regulator of host metabolism, immune homeostasis, and neuroendocrine function. Growing evidence indicates that endurance exercise can influence both the composition and functional activity of the intestinal microbiota. However, most available studies are cross-sectional, focus on extreme endurance athletes, and rarely integrate high-resolution metagenomic data with physiological and nutritional parameters. Data on competitive middle- and long-distance runners remain limited.

This pilot study originates from a real-world need emerging in competitive athletics, where endurance athletes frequently present with gastrointestinal disturbances, recurrent upper respiratory tract infections (URTIs), high physical and psychological stress, and dietary patterns primarily structured around energy requirements rather than microbiota-supportive strategies. The project was therefore designed as a multidisciplinary intervention integrating sports nutrition, microbiota analysis, and performance monitoring.

Two competitive male middle- and long-distance runners, identified as potential candidates for the 2028 Olympic Games, were enrolled and monitored over a 16-week period. An initial gut microbiota assessment was performed at the beginning of the project (early February) using a shotgun metagenomic platform (Wellmicro®, Bologna), enabling comprehensive multi-kingdom profiling (bacteria, fungi, viruses, and parasites), high-resolution taxonomic characterization, and functional inference based on microbial gene content and enzyme-associated metabolic pathways.

This approach allowed the evaluation of ecological indices (e.g., alpha- and beta-diversity), identification of health-relevant microbial strains, and estimation of the microbiota's contribution to key metabolic functions, including short-chain fatty acid production, amino acid metabolism (e.g., tryptophan derivatives), and pathways related to gut barrier integrity and host–microbiota interaction axes. Microbiota data were integrated with clinical evaluation, body composition (bioelectrical impedance analysis, anthropometry, and skinfolds), lifestyle

factors, and training-related parameters. Based on these findings, a personalized nutritional and nutraceutical strategy was implemented, aiming to modulate microbial composition and function while maintaining alignment with sport-specific energy demands. The intervention was developed within a multidisciplinary framework involving sports nutritionists, coaches, and other professionals.

At the time of writing, the study is ongoing and follow-up microbiota sampling is planned for May, in alignment with the athletes' competitive calendar. This represents a relevant methodological challenge, as elite athletes are rarely available during fully controlled, competition-free periods, highlighting the importance of real-world study designs in sports science.

This study represents the preliminary phase of a broader research project aimed at investigating whether targeted nutritional strategies can modulate gut microbiota composition and functional potential in endurance athletes, and how these changes may relate to metabolic adaptation, gastrointestinal tolerance, immune resilience, and performance outcomes.

Despite the limited sample size and ongoing design, this pilot investigation provides a clinically relevant framework for integrating advanced metagenomic microbiota analysis into sports practice, supporting the development of personalized, microbiota-oriented nutritional strategies within elite athlete management.

Abstract (versione in italiano)

Il microbiota intestinale è sempre più riconosciuto come un regolatore chiave del metabolismo dell'ospite, dell'omeostasi immunitaria e della funzione neuroendocrina. Evidenze crescenti indicano che l'esercizio di endurance può influenzarne composizione e attività funzionale. Tuttavia, la maggior parte degli studi è di tipo trasversale, focalizzata su atleti di endurance estremo e raramente integra dati metagenomici ad alta risoluzione con parametri fisiologici e nutrizionali. I dati su mezzofondisti e fondisti competitivi restano limitati.

Il presente studio pilota nasce da un'esigenza concreta dell'atletica competitiva, dove gli atleti di endurance presentano frequentemente disturbi gastrointestinali, infezioni ricorrenti delle vie respiratorie superiori, elevati livelli di stress e pattern alimentari orientati soprattutto alle richieste energetiche piuttosto che al supporto del microbiota. Il progetto è stato quindi concepito come un intervento multidisciplinare che integra nutrizione sportiva, analisi del microbiota e monitoraggio della performance.

Due atleti maschi competitivi di mezzofondo e fondo, candidati per i Giochi Olimpici 2028, sono stati arruolati e monitorati per 16 settimane. Una valutazione iniziale del microbiota intestinale è stata effettuata a inizio febbraio mediante piattaforma metagenomica shotgun (Wellmicro®, Bologna), permettendo una profilazione multi-regno (batteri, funghi, virus e parassiti), una caratterizzazione tassonomica ad alta risoluzione e un'inferenza funzionale basata sul contenuto genico e sui pathway metabolici.

Questo approccio ha consentito la valutazione di indici ecologici (diversità alfa e beta), l'identificazione di ceppi rilevanti per la salute e la stima del contributo del microbiota a funzioni metaboliche chiave, tra cui produzione di acidi grassi a catena corta, metabolismo degli amminoacidi (es. derivati del triptofano) e pathway legati all'integrità della barriera intestinale e alle interazioni ospite–microbiota.

I dati del microbiota sono stati integrati con valutazioni cliniche, composizione corporea (bioimpedenziometria, antropometria e plicometria), stile di vita e parametri di allenamento. Su questa base è stata implementata una strategia

nutrizionale e nutraceutica personalizzata, con l'obiettivo di modulare il microbiota mantenendo l'aderenza alle richieste energetiche della disciplina. L'intervento è stato sviluppato in un framework multidisciplinare con nutrizionisti sportivi, allenatori e altri professionisti.

Al momento della stesura, lo studio è in corso e un follow-up del microbiota è previsto per maggio, in accordo con il calendario agonistico. Ciò rappresenta una sfida metodologica, poiché gli atleti di alto livello sono raramente disponibili in condizioni completamente controllate, evidenziando l'importanza di studi in contesti reali.

Questo lavoro rappresenta la fase preliminare di un progetto più ampio volto a verificare se strategie nutrizionali mirate possano modulare composizione e funzione del microbiota negli atleti di endurance e come tali modifiche si correlino con adattamento metabolico, tolleranza gastrointestinale, resilienza immunitaria e performance.

Nonostante la limitata numerosità campionaria e il disegno ancora in corso, questa indagine pilota fornisce un framework clinicamente rilevante per l'integrazione di analisi metagenomiche avanzate nella pratica sportiva.

Sintesi estesa

1. Introduzione

Negli ultimi due decenni, il microbiota intestinale ha acquisito un ruolo centrale nella comprensione della fisiologia umana, fino a essere considerato un vero e proprio organo metabolico ed endocrino. Questo ecosistema complesso, costituito da trilioni di microrganismi tra cui batteri, virus, funghi e archea, è coinvolto in una vasta gamma di funzioni biologiche essenziali, tra cui la regolazione del metabolismo energetico, il mantenimento dell'omeostasi immunitaria, la protezione della barriera intestinale e la modulazione della comunicazione tra sistema intestinale e sistema nervoso centrale.

Uno degli aspetti più rilevanti del microbiota riguarda la sua capacità di produrre metaboliti bioattivi, come gli acidi grassi a corta catena (short-chain fatty acids, SCFA), derivanti dalla fermentazione delle fibre alimentari non digeribili. Tra questi, il butirrato rappresenta una fonte energetica primaria per gli enterociti e svolge un ruolo fondamentale nel mantenimento dell'integrità della mucosa intestinale, mentre acetato e propionato partecipano alla regolazione del metabolismo sistemico, influenzando processi come la gluconeogenesi e il metabolismo lipidico.

Nel contesto dell'attività fisica, e in particolare negli sport di endurance, il microbiota intestinale assume un ruolo ancora più complesso e dinamico. L'esercizio fisico prolungato comporta infatti una serie di adattamenti fisiologici che coinvolgono diversi sistemi, tra cui quello cardiovascolare, metabolico, immunitario e gastrointestinale. Durante l'attività, la ridistribuzione del flusso ematico verso i muscoli attivi determina una riduzione della perfusione intestinale, condizione che può compromettere l'integrità della barriera epiteliale e favorire l'insorgenza di sintomi gastrointestinali.

Questi disturbi, che includono dolore addominale, gonfiore, diarrea e nausea, sono estremamente frequenti negli atleti di endurance e possono avere un impatto diretto sulla performance. Inoltre, l'aumento della permeabilità intestinale può facilitare il passaggio di componenti batterici nel circolo sistemico, contribuendo a uno stato di infiammazione di basso grado.

Parallelamente, l'esercizio intenso può influenzare il sistema immunitario. In particolare, dopo sforzi prolungati si osserva una fase temporanea di immunosoppressione, nota come "finestra aperta" ("open window"), durante la quale aumenta la suscettibilità alle infezioni, soprattutto a carico delle vie respiratorie. In questo contesto, il microbiota intestinale emerge come un potenziale modulatore della risposta immunitaria, contribuendo alla regolazione dei processi infiammatori e alla protezione dell'organismo.

Un ulteriore elemento di interesse è rappresentato dall'asse intestino-cervello, attraverso il quale il microbiota è in grado di influenzare la regolazione dello stress, la risposta neuroendocrina e alcuni aspetti cognitivi e comportamentali. I metaboliti microbici, infatti, possono modulare la produzione di neurotrasmettitori e interagire con il sistema nervoso attraverso vie neurali e ormonali, suggerendo un possibile ruolo del microbiota anche nella regolazione della fatica centrale e della resilienza allo stress.

Nonostante il crescente numero di evidenze scientifiche, la maggior parte degli studi disponibili presenta limiti significativi. Molti lavori sono di tipo trasversale, non permettono di stabilire relazioni causali e si basano su metodiche di analisi con risoluzione limitata. Inoltre, l'attenzione è spesso rivolta ad atleti di ultra-endurance, mentre risultano meno indagati gli atleti di mezzofondo e fondo, che rappresentano un modello particolarmente interessante per la combinazione di metabolismo aerobico e anaerobico.

Alla luce di queste considerazioni, emerge la necessità di studi longitudinali, condotti in contesti reali e basati su approcci integrati, capaci di mettere in relazione microbiota, nutrizione, allenamento e performance.

2. Obiettivi dello studio

Il presente lavoro si inserisce in questo contesto con l'obiettivo di approfondire il ruolo del microbiota intestinale negli atleti di endurance, adottando un approccio multidisciplinare e longitudinale.

L'obiettivo principale dello studio è quello di caratterizzare il profilo composizionale e funzionale del microbiota intestinale in atleti di mezzofondo e fondo, analizzandone le potenziali implicazioni nei processi di adattamento

fisiologico all'allenamento. In particolare, lo studio si propone di identificare specifici pattern microbici associati alla performance e alla capacità di adattamento allo stress fisico.

Un ulteriore obiettivo riguarda la valutazione degli effetti di un intervento nutrizionale personalizzato sulla modulazione del microbiota. L'ipotesi alla base è che modifiche mirate della dieta, associate eventualmente a strategie di integrazione, possano influenzare non solo la composizione microbica, ma anche la sua attività metabolica e le interazioni con l'organismo ospite.

Lo studio si propone inoltre di esplorare le possibili relazioni tra microbiota e variabili cliniche e prestative, tra cui sintomi gastrointestinali, stato immunitario, composizione corporea e parametri di allenamento. Questo approccio integrato consente di superare una visione riduttiva del microbiota, considerandolo invece come parte di un sistema complesso in cui interagiscono molteplici fattori.

Infine, il lavoro si inserisce in un progetto più ampio volto a sviluppare strategie nutrizionali personalizzate basate sul microbiota, con l'obiettivo di migliorare la salute e la performance degli atleti in contesti reali.

3. Materiali e metodi

Lo studio è stato progettato come un'indagine longitudinale della durata di 16 settimane, condotta in un contesto reale di allenamento sportivo, al fine di garantire una maggiore validità ecologica dei risultati.

Sono stati arruolati due atleti maschi di alto livello, impegnati in discipline di mezzofondo e fondo, con caratteristiche fisiche e prestative comparabili, ma con differenze rilevanti in termini di storia clinica, abitudini alimentari e condizioni di salute.

La fase iniziale dello studio ha previsto una valutazione approfondita dello stato di salute, dello stile di vita e delle abitudini nutrizionali dei partecipanti. Sono stati raccolti dati relativi alla storia clinica, alla presenza di sintomi gastrointestinali, all'utilizzo di farmaci e integratori e alle abitudini alimentari, con particolare attenzione alla qualità della dieta e alla varietà degli alimenti consumati.

La composizione corporea è stata valutata mediante bioimpedenziometria, mentre i parametri di allenamento sono stati monitorati attraverso dispositivi wearable e registrazioni su diario, che includevano informazioni su carico di lavoro, recupero, sintomi e benessere generale.

L'analisi del microbiota intestinale è stata effettuata mediante tecnologia di shotgun metagenomics, che rappresenta uno degli approcci più avanzati attualmente disponibili. Questa metodica consente non solo di identificare i microrganismi presenti fino al livello di specie, ma anche di analizzare il potenziale funzionale del microbiota, attraverso la valutazione dei geni coinvolti in specifici pathway metabolici.

Sulla base dei risultati ottenuti nella fase iniziale, è stato sviluppato un intervento nutrizionale personalizzato, elaborato in collaborazione con il nutrizionista sportivo degli atleti. L'intervento ha previsto modifiche qualitative della dieta, finalizzate a migliorare la diversità alimentare, aumentare l'apporto di fibre e ridurre il consumo di alimenti ultra-processati, oltre all'introduzione di strategie di integrazione mirata, in funzione delle caratteristiche individuali.

Durante il periodo di intervento, gli atleti sono stati monitorati attraverso visite di follow-up e registrazioni nel diario, al fine di valutare l'aderenza alle indicazioni e raccogliere informazioni utili per l'interpretazione dei risultati.

4. Risultati

L'analisi dei dati ha evidenziato la presenza di differenze significative tra i due atleti, nonostante la similarità dei carichi di allenamento e del livello competitivo.

Dal punto di vista della composizione del microbiota, entrambi i soggetti presentavano una predominanza dei phyla Bacteroidetes e Firmicutes, in linea con quanto riportato nella letteratura. Tuttavia, a livello di specie e di organizzazione ecologica, emergevano configurazioni profondamente diverse.

Nel primo atleta si osservava una forte dominanza di Segatella copri, che rappresentava oltre la metà della comunità batterica. Questa configurazione era associata a una ridotta diversità e a una distribuzione poco uniforme delle

specie, suggerendo un microbiota altamente specializzato e orientato verso il metabolismo dei carboidrati.

Al contrario, il secondo atleta presentava una maggiore diversità microbica e una distribuzione più equilibrata delle specie, senza la predominanza di un singolo taxon. Questo profilo indicava una struttura più omogenea e potenzialmente più resiliente.

Dal punto di vista funzionale, entrambi i microbioti mostravano una buona capacità di produzione di acetato e propionato, ma una ridotta potenzialità nella sintesi di butirrato. Questo dato è particolarmente rilevante, considerando il ruolo centrale del butirrato nel mantenimento della salute intestinale e nella modulazione della risposta infiammatoria.

L'analisi delle abitudini alimentari ha evidenziato in entrambi gli atleti una dieta caratterizzata da un elevato consumo di carboidrati, ma con una scarsa varietà e un ridotto apporto di fibre, in particolare nel primo atleta. Questo aspetto potrebbe aver contribuito alla configurazione del microbiota osservata.

Per quanto riguarda l'intervento nutrizionale, i due atleti hanno mostrato livelli di aderenza molto differenti. Il primo atleta ha introdotto modifiche significative, aumentando il consumo di alimenti ricchi di fibre, migliorando la varietà della dieta e ottimizzando l'idratazione. Tali cambiamenti si sono associati a un miglioramento dei sintomi gastrointestinali e della tolleranza all'esercizio.

Il secondo atleta, invece, ha mostrato una scarsa aderenza alle indicazioni nutrizionali, mantenendo abitudini alimentari sostanzialmente invariate. Questo si è riflesso in una mancata osservazione di miglioramenti significativi, sia dal punto di vista clinico sia funzionale.

5. Discussione e conclusioni

I risultati di questo studio evidenziano come il microbiota intestinale negli atleti di endurance possa assumere configurazioni differenti, anche in presenza di stimoli allenanti simili, suggerendo l'esistenza di molteplici strategie adattative.

Il confronto tra i due atleti mette in luce due modelli distinti: da un lato, un microbiota altamente specializzato e dominato da un singolo taxon,

potenzialmente efficiente ma meno resiliente; dall'altro, un microbiota più diversificato ed equilibrato, ma non necessariamente associato a migliori condizioni cliniche o prestative.

Questi risultati sottolineano come la diversità microbica, pur rappresentando un indicatore importante di salute, non sia di per sé sufficiente a garantire un funzionamento ottimale, ma debba essere interpretata all'interno di un contesto più ampio che include fattori nutrizionali, immunitari e ambientali.

Un elemento particolarmente rilevante emerso dallo studio riguarda il ruolo dell'alimentazione. La dieta si conferma come uno dei principali determinanti della composizione e della funzione del microbiota, influenzando la disponibilità di substrati per il metabolismo microbico e la produzione di metaboliti bioattivi.

In questo senso, l'intervento nutrizionale ha mostrato effetti positivi nel caso in cui sia stato effettivamente seguito, evidenziando l'importanza dell'aderenza come fattore chiave per il successo di strategie personalizzate. Al contrario, la mancata adesione rappresenta un limite significativo, che riduce la possibilità di osservare effetti concreti e di trarre conclusioni definitive.

Lo studio mette inoltre in evidenza la necessità di un approccio integrato, che consideri il microbiota come parte di un sistema complesso in cui interagiscono fattori biologici, comportamentali e ambientali. In questo contesto, la personalizzazione degli interventi emerge come un elemento fondamentale, sia per migliorare la salute degli atleti sia per ottimizzare la performance.

Nonostante i limiti legati al ridotto numero di partecipanti e alla natura preliminare dello studio, i risultati ottenuti forniscono indicazioni importanti per future ricerche. In particolare, sarà necessario ampliare il campione, completare il follow-up microbiologico e approfondire le relazioni tra microbiota, dieta e performance.

In conclusione, questo lavoro contribuisce a rafforzare l'idea che il microbiota intestinale rappresenti un potenziale modulatore dell'adattamento fisiologico all'esercizio di endurance e che strategie nutrizionali mirate possano rappresentare uno strumento promettente per il miglioramento della salute e della performance degli atleti.

1. Introduction

1.1 The gut microbiota as a metabolic and endocrine organ

Over the last two decades, the human gut microbiota has emerged as a central regulator of host physiology (Belkaid and Hand 2014; Sonnenburg and Bäckhed 2016). It is now recognized as a dynamic and metabolically active ecosystem functioning as a virtual organ, contributing to energy metabolism, immune regulation, intestinal barrier integrity, and neuroendocrine signaling (Belkaid and Hand 2014; Sonnenburg and Bäckhed 2016; Koh et al. 2016).

The gut microbiota comprises trillions of microorganisms, including bacteria, archaea, viruses, and fungi. At the bacterial level, the dominant phyla include Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia (Sonnenburg and Bäckhed 2016). Beyond taxonomic composition, the gut microbiome encompasses not only the microorganisms themselves but also their collective genetic content, functional activity, and interactions with the surrounding environment (Sonnenburg and Bäckhed 2016; Pedersen et al. 2016). This integrated system provides metabolic capabilities that the host alone cannot perform, including the fermentation of non-digestible carbohydrates, production of bioactive metabolites, modulation of bile acids, and synthesis of vitamins and neurotransmitter precursors (Koh et al. 2016; Pedersen et al. 2016). Among microbial metabolites, short-chain fatty acids (SCFAs)—primarily acetate, propionate, and butyrate—play a central role in host physiology (Koh et al. 2016; Louis and Flint 2017). Butyrate serves as the primary energy substrate for colonocytes and contributes to the maintenance of intestinal epithelial integrity by reinforcing tight junctions and promoting mucosal repair (Koh et al. 2016; Louis and Flint 2017). Acetate and propionate enter systemic circulation and participate in hepatic gluconeogenesis, lipid metabolism, and peripheral metabolic signaling (Louis and Flint 2017). Collectively, SCFAs exert anti-inflammatory effects, modulate regulatory T-cell (Treg) activity, and influence systemic immune homeostasis (Belkaid and Hand 2014; Koh et al. 2016).

Beyond metabolic regulation, the gut microbiota is deeply integrated with the host neuroendocrine system through the gut–brain axis (Cryan et al. 2019).

Microbial metabolites such as SCFAs, indole derivatives from tryptophan metabolism, gamma-aminobutyric acid (GABA), and serotonin precursors can influence vagal signaling, hypothalamic–pituitary–adrenal (HPA) axis activity, stress responsiveness, and cognitive function (Cryan et al. 2019; Agus et al. 2018; Strandwitz et al. 2019). This bidirectional communication network suggests that alterations in microbial composition or function may affect not only gastrointestinal (GI) health but also systemic stress adaptation and performance-related outcomes.

Importantly, health is not associated with a single “ideal” microbial composition. Rather, a range of configurations compatible with eubiosis exists, reflecting inter-individual variability shaped by genetics, diet, training load, environmental exposures, and medical history (Sonnenburg and Bäckhed 2016). Dysbiosis, defined as a disruption of microbial equilibrium or functional output, has been associated with chronic low-grade inflammation, impaired barrier integrity, metabolic inefficiency, and altered stress responses (Belkaid and Hand 2014; Sonnenburg and Bäckhed 2016).

1.2 Endurance Exercise and Gastrointestinal Stress

Endurance exercise imposes substantial physiological stress on multiple organ systems, including the GI tract. During prolonged or high-intensity exercise, blood flow is preferentially redistributed toward skeletal muscle and peripheral circulation, resulting in relative splanchnic hypoperfusion. This ischemic condition, particularly when combined with dehydration, thermal stress, and heightened sympathetic activation, can compromise epithelial integrity and increase intestinal permeability (Costa et al. 2017).

Exercise-induced GI symptoms are highly prevalent among endurance athletes, with reported incidence ranging from 30% to 70% in runners and cyclists, and up to 90% in ultra-endurance events (Costa et al. 2017; Jeukendrup 2011). Common manifestations include abdominal cramping, bloating, diarrhea, nausea, and urgency. Beyond discomfort, these symptoms may directly impair performance, alter pacing strategies, and increase the likelihood of withdrawal from competition.

The pathophysiology of exercise-induced GI syndrome is multifactorial, involving reduced intestinal perfusion, sympathetic nervous system activation, increased cortisol levels, mechanical stress due to repetitive movement, and suboptimal nutritional practices (Costa et al. 2017; Jeukendrup 2011). Increased intestinal permeability may facilitate the translocation of bacterial components such as lipopolysaccharide (LPS), contributing to systemic low-grade inflammation and exercise-associated endotoxemia (Costa et al. 2017).

In this context, the gut microbiota emerges as a potential key modulator of GI resilience and individual susceptibility to exercise-induced stress. A stable and functionally diverse microbial ecosystem may support epithelial barrier integrity, modulate inflammatory responses, and improve tolerance to high carbohydrate availability during prolonged exercise (Costa et al. 2017). Conversely, alterations in microbial composition or function (dysbiosis) may predispose athletes to intestinal permeability, exaggerated inflammatory responses, impaired nutrient handling, and great severity of GI symptoms.

1.3 Microbiota and Metabolic Adaptation in Middle- and Long-Distance Athletes

Middle- and long-distance running requires a complex integration of metabolic capacities. Events such as 800 m and 1500 m are characterized by a substantial contribution of anaerobic glycolysis and rapid lactate accumulation, whereas longer distances (5000 m, 10000 m, and half-marathon) predominantly rely on oxidative metabolism, mitochondrial efficiency, and substrate utilization flexibility. Athletes competing in these disciplines must therefore optimize both glycolytic flux and aerobic capacity to sustain performance across varying intensities.

Recent evidence suggests that the gut microbiota may contribute to metabolic adaptation to exercise. Observational studies have reported that endurance athletes exhibit greater microbial diversity and enrichment of SCFA-producing taxa compared to sedentary individuals (Clarke et al. 2014; Barton et al. 2018; Petersen et al. 2017). These microbial features have been associated with improved metabolic flexibility, enhanced oxidative capacity, and favorable performance-related markers such as VO_2max. However, most available data

remain associative, and the mechanistic pathways linking microbiota composition to exercise performance are still under investigation (Clarke et al. 2014; Barton et al. 2018).

Of particular interest is the genus *Veillonella*, an anaerobic bacterium capable of metabolizing lactate into propionate. Lactate, once considered merely a metabolic byproduct, is now recognized as a key intermediate in the lactate shuttle, acting both as a fuel substrate and a signaling molecule involved in metabolic regulation. Experimental evidence has shown that *Veillonella* abundance may increase following endurance exercise and that the conversion of exercise-derived lactate into propionate may contribute to enhanced endurance capacity (Scheiman et al. 2019). Although causality remains to be fully established, these findings suggest a potential bidirectional interaction between host metabolism and microbial function.

In this context, middle-distance runners – who are exposed to repeated elevations in systemic lactate levels – represent a particularly interesting model for investigating host–microbiota metabolic interactions. The interplay between lactate production, microbial lactate utilization, and downstream metabolite generation may represent a novel mechanism contributing to metabolic efficiency and performance adaptation, although this area remains largely unexplored.

1.4 Microbiota, Immune Resilience, and the “Open Window” Theory

Endurance training exerts significant effects also on immune function. While moderate exercise is associated with enhanced immune surveillance; prolonged or high-intensity exercise may induce a transient immunosuppression. The “open window” theory describes a period of approximately 3–72 hours following strenuous exercise during which athletes may exhibit increased susceptibility to upper respiratory tract infections (URTIs). This phenomenon is characterized by reduced natural killer (NK) cell activity, altered T-cell responses, decreased mucosal IgA secretion, and elevated cortisol levels (Nieman and Wentz 2019).

Nutritional status plays a critical role in modulating immune responses during and after exercise. In particular, low carbohydrate availability, reduced glycogen stores, and inadequate energy intake may exacerbate cortisol responses and

promote inflammatory signaling. Within this framework, the gut microbiota has emerged as an additional regulator of immune resilience. Microbial derived metabolites, such as SCFAs, contribute to the regulation of immune homeostasis by promoting Treg differentiation, enhancing mucosal barrier function, and modulating inflammatory pathways. Furthermore, reduced microbial diversity and altered microbial composition have been associated with increased susceptibility to infections (Nieman and Wentz 2019).

Interventional studies investigating probiotic supplementation in athletes have reported reductions in GI symptoms and URTIs incidence, along with modulation of inflammatory markers and tryptophan–kynurenine metabolism. However, results remain heterogeneous, and the variability in probiotic strains, dosages, and study designs limits the generalizability of findings. These observations nevertheless support the hypothesis that the gut microbiota may play a role in shaping immune adaptation to training-related stress (Lamprecht et al. 2012; Pugh et al. 2019; West et al. 2009). In this context, the interaction between training load, nutritional strategies, and microbiota composition may represent a key determinant of immune resilience in endurance athletes, with potential implications for both health and performance.

1.5 The Gut–Brain Axis, Stress Regulation, and Neuroendocrine Adaptation

Chronic training stress, insufficient recovery, and low energy availability can lead to maladaptive states such as non-functional overreaching and overtraining syndrome (OTS). These conditions are characterized by persistent fatigue, impaired performance, mood disturbances, and dysregulation of the HPA axis (Meeusen et al. 2013).

The gut–brain axis represents a mechanistic bridge between intestinal ecology and neuroendocrine regulation (Cryan et al. 2019). In this context, exercise-induced stress has been proposed to interact with the gut–microbiota–brain axis, influencing not only physiological responses but also behavioral and psychological adaptations in athletes. Evidence suggests that physical stress, dietary factors and gut microbiota composition may collectively modulate central nervous system activity and stress-related behaviors supporting a

bidirectional relationship between exercise load and neuroendocrine function (Clark and Mach 2016).

Recent experimental findings further support this concept, indicating that specific microbial species, such as *Eubacterium rectale* and *Coprococcus eutactus*, may influence exercise performance through gut–brain axis mechanisms, including the modulation of dopaminergic signaling (Dohnalová et al. 2022). However, the translation of these findings to human physiology and athletic performance remains to be fully elucidated.

Microbial metabolites and signaling molecules can influence vagal activity, cortisol secretion, neurotransmitter synthesis, and stress perception (Cryan et al. 2019; Agus et al. 2018; Strandwitz et al. 2019). In particular, microbial involvement in tryptophan metabolism and GABA production suggests potential pathways through which the microbiota may modulate central fatigue, mood regulation, and stress resilience (Agus et al. 2018; Strandwitz et al. 2019). Human studies investigating psychobiotic interventions suggest that specific probiotic strains may attenuate cortisol responses to stress, modulate brain activity patterns, and improve cognitive performance under stress conditions (Messaoudi et al. 2011; Tillisch et al. 2013; Allen et al. 2016). However, heterogeneity in study design and intervention protocols limits the generalizability of these findings.

Although direct evidence linking gut microbiota to OTS in athletes is currently limited, converging evidence from exercise physiology and microbiome research suggest that microbial composition and function may contribute to neuroendocrine adaptation to chronic training load (Cryan et al. 2019; Meeusen et al. 2013).

Taken together, these findings support the hypothesis that gut microbiota may play a role in both physiological and psychological adaptation to endurance training, with potential implications for performance, recovery, and stress resilience.

2. Aim of the Thesis

Despite growing evidence linking the gut microbiota to exercise physiology, several important gaps remain. Most available studies are cross-sectional, focus predominantly on elite marathoners or ultra-endurance athletes, or rely on 16S rRNA sequencing, which provides limited functional resolution (Clarke et al. 2014; Barton et al. 2018; Petersen et al. 2017). Furthermore, few investigations adopt an integrative and longitudinal approach combining microbial composition, functional metabolic potential, nutritional strategies, and performance-related physiological parameters within real-world athletic settings.

In competitive practice, endurance athletes frequently experience gastrointestinal disturbances, recurrent upper respiratory tract infections, high physical and psychological stress, and dietary patterns primarily structured around energy requirements rather than microbiota-supportive strategies. These factors highlight the need for a more comprehensive and multidisciplinary approach integrating microbiota-targeted interventions into athlete management.

Competitive middle- and long-distance runners represent a particularly relevant model due to their complex metabolic demands, characterized by repeated exposure to high lactate flux alongside sustained aerobic stress. Investigating both the compositional and functional features of their gut microbiota may provide insights into key processes such as metabolic adaptation, gastrointestinal tolerance, immune resilience, and stress regulation.

In this context, the present pilot study was designed as part of an ongoing multidisciplinary project involving sports nutrition, performance coaching, and microbiota analysis, carried out in collaboration with Atletica Reggio, a track and field association based in Reggio Emilia with over 1,200 registered athletes. An initial gut microbiota assessment was performed at the beginning of the intervention period, using a high-resolution shotgun metagenomic approach, to characterize microbial composition and functional metabolic potential under real training conditions. Based on these findings, a personalized nutritional and

nutraceutical strategy was implemented, with the aim of modulating microbial composition and function while maintaining alignment with sport-specific energy and performance requirements. Follow-up microbiota sampling is planned after the intervention period, in accordance with the athletes' competitive calendar, to evaluate potential changes in microbial structure and functional output.

Within this framework, the aim of the present thesis is to investigate the composition and functional potential of the gut microbiota in competitive middle- and long-distance runners, with particular attention to the identification of microbial taxa or signatures potentially associated with endurance training and athletic performance. The study also seeks to evaluate longitudinal changes in the gut microbiota following a personalized nutritional and supplementation intervention over a period of approximately 2–3 months, assessing variations in microbial diversity, taxonomic composition, and predicted metabolic pathways.

Furthermore, this work aims to explore potential associations between gut microbiota profiles—both at the compositional and functional level—and performance-related parameters, including physiological indicators, training load variables, and body composition metrics. Through the integration of metagenomic, clinical, nutritional, and performance data, the thesis ultimately aims to contribute to a better understanding of the gut microbiota as a potential modulator of metabolic adaptation, immune function, stress resilience, and performance in endurance athletes.

3. Materials and Methods

3.1 Study Design

This study was designed as a 16-week longitudinal pilot investigation conducted in a real-world athletic setting, aimed at evaluating gut microbiota composition and functional potential in competitive middle- and long-distance runners, as well as exploring potential changes following a personalized nutritional and supplementation intervention.

An initial clinical, lifestyle, and nutritional assessment was performed at the first study visit. Gut microbiota sampling was subsequently carried out within a variable time window (9–17 days after the initial visit), reflecting practical constraints related to athlete availability and training schedules. Therefore, the initial microbiota assessment represents a real-world baseline under ongoing training conditions rather than a strictly controlled pre-intervention time point.

Following the availability of microbiota results, a personalized nutritional and supplementation strategy was implemented in collaboration with the athletes' primary sports nutritionist, ensuring consistency with sport-specific dietary requirements and performance goals.

An intermediate follow-up visit was initially planned approximately 4 weeks after the start of the intervention to monitor early responses. However, due to variability in participant compliance and scheduling constraints, this visit was conducted after approximately 6 weeks in Athlete A and 7 weeks in Athlete B.

Participants were monitored throughout the study period using structured follow-up visits and an athlete diary, which included the recording of training load, recovery status, and relevant lifestyle factors. A follow-up gut microbiota assessment is planned after the intervention period (May), in accordance with the athletes' competitive calendar, to evaluate potential changes in microbial composition and functional output. According to the study timeline outlined in the project protocol (Figure 1), the study included an initial assessment phase, an intervention period with intermediate monitoring, and a planned post-intervention microbiota evaluation.

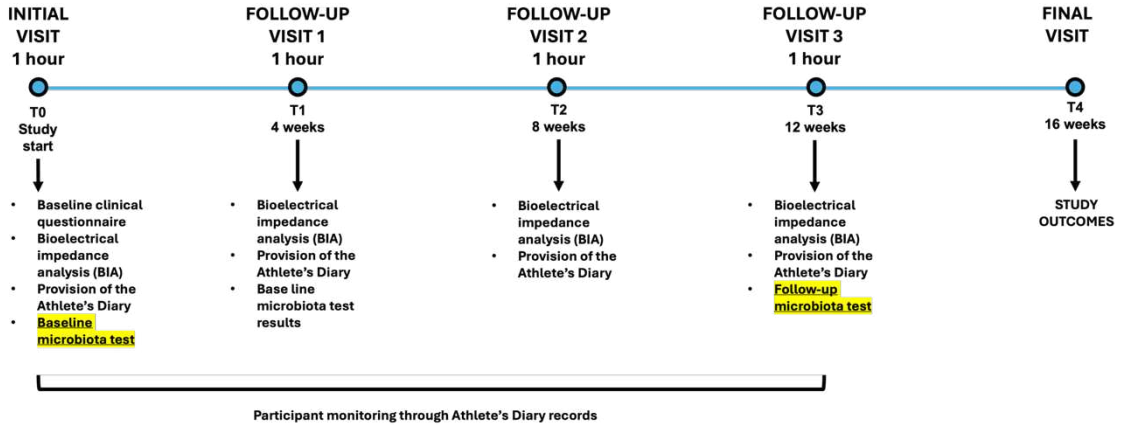

Figure 1 – Study timeline and project protocol.

3.2 Participants

Two male competitive middle- and long-distance runners aged 23 and 29 years were enrolled in the study. Athlete A, although born and raised in Italy, has a Moroccan family background, which may be relevant in terms of early-life environmental and dietary exposures potentially influencing gut microbiota composition.

Both athletes competed at high national and international levels; Athlete A had participated in the 2024 Olympic Games, and both were considered potential candidates for future Olympic qualification.

Inclusion criteria were defined according to the research consent document (see Appendix A) and included:

- age between 20 and 35 years,
- participation in endurance disciplines (middle- and long-distance running),
- good general health status,
- absence of clinically relevant diagnosed conditions,
- availability for the entire duration of the study.

All participants provided written informed consent prior to enrollment. Data were anonymized and handled in compliance with General Data Protection Regulation (GDPR) regulations, as specified in the consent documentation.

3.3 Baseline Clinical and Lifestyle Assessment

All participants completed a structured anamnesis questionnaire prior to the start of the study, with the aim of collecting baseline information on health status, lifestyle habits, and dietary patterns. The full structure of the questionnaire is reported in Appendix B.

The questionnaire included sections on demographic characteristics, medical history and previous diagnoses, GI symptoms and bowel habits, history of antibiotic use, current medications and dietary supplements, as well as habitual dietary patterns and lifestyle factors including sleep quality, perceived stress, and physical activity.

Particular attention was given to dietary supplement use. Participants were asked to report all regularly used supplements, including sport-specific products (e.g., carbohydrate gels, protein supplements), micronutrients, and other nutraceutical compounds. For each product, information regarding type, frequency, duration of use and timing in relation to training sessions was collected for each athlete.

In addition to active compounds, the potential presence of additives, emulsifiers and other excipients commonly found in processed supplements was considered, given their possible influence on gut microbiota composition and intestinal barrier function. Although a quantitative assessment of these components was not performed, their presence was qualitatively considered as a potential environmental factor in the interpretation of microbiota data.

No standardized supplementation protocol was imposed at baseline, and athletes maintained their habitual supplementation practices unless otherwise specified during the intervention phase.

Dietary habits were assessed through a detailed self-reported questionnaire exploring habitual intake of major food groups (e.g., carbohydrates, fruits, vegetables, animal products, dairy products, legumes, and fats), as well as hydration status and consumption of stimulants or alcohol.

This information were used to identify potential factors influencing gut microbiota composition and to contextualize microbiota results within the participants' nutritional and lifestyle background.

3.4 Nutritional and Supplementation Intervention

At the beginning of the study, participants were already following structured dietary plans supervised by a sports nutritionist. Following the initial gut microbiota assessment, the nutritional protocol was adjusted in agreement with the primary nutritionist ensuring consistency with sport-specific energy requirements and ongoing performance-oriented strategies.

Baseline supplementation habits were carefully reviewed and taken into account when designing the intervention, particularly in relation to GI tolerance, immune support and training-related demands.
The intervention included:

- dietary adjustments aimed at supporting gut microbiota modulation, with particular attention to food quality, diversity, and intake of microbiota-relevant substrates,
- targeted supplementation strategies, including probiotics, prebiotics, and selected nutraceutical compounds when indicated,
- monitoring of adherence and lifestyle factors through athlete diaries.

The intervention period lasted approximately 12 weeks. A follow-up gut microbiota assessment is planned after the intervention phase, in accordance with the athletes' competitive calendar, to evaluate potential changes in microbial composition and functional output.

3.5 Gut Microbiota Sampling and Analysis

Fecal samples were collected at the beginning of the study using a standardized stool collection kit (Wellmicro Gut Plus®, Wellmicro S.r.l., Italy), according to the manufacturer's protocol. Participants collected samples at home using sterile collection devices and a stabilizing buffer to preserve microbial DNA integrity. Samples were subsequently shipped to a certified external laboratory under controlled conditions.

A follow-up fecal sample collection is planned after the intervention period (approximately 12 weeks), in accordance with the athletes' competitive calendar, to evaluate potential changes in gut microbiota composition and functional output.

Microbial DNA extraction was performed using validated protocols optimized for complex fecal matrices. Whole-metagenome shotgun sequencing was conducted using high-throughput next-generation sequencing (NGS) platforms, enabling comprehensive taxonomic profiling at species-level resolution and functional gene characterization of microbial gene content.

Shotgun metagenomics was selected over 16S rRNA sequencing due to its higher taxonomic resolution and its ability to provide direct insight into microbial functional potential.

Bioinformatic processing was carried out using a proprietary computational pipeline, including quality control and filtering of raw sequencing reads, host DNA removal, taxonomic assignment based on curated microbial genome databases, functional gene annotation, and reconstruction of predicted metabolic pathways.

Taxonomic outputs were reported as relative abundance (%) at phylum, genus, and species levels.

Functional analysis included prediction of SCFA production potential (including acetate, propionate, butyrate), lactate utilization pathways, tryptophan metabolism and indole derivatives, GABA production, serotonin precursor metabolism, gas production (e.g., methane), β-glucuronidase (GUS) activity, indices related to intestinal barrier integrity, gut–brain axis-related functional modules.

Functional predictions were based on the relative abundance of genes involved in specific metabolic pathways, quantified through the proprietary algorithm developed by the laboratory.

3.6 Body Composition Assessment

Body composition was assessed at beginning of the study and monitored during throughout the intervention period.

Bioelectrical impedance analysis (BIA) was performed monthly using a BioTekna ACC device (BioTekna, Italy). Measurements were conducted under standardized conditions by the investigator, including controlled hydration status and consistent timing relative to training sessions, when feasible.
Anthropometric measurements and skinfold thickness assessment were performed quarterly by the supervising sports nutritionist, following standard anthropometric procedures.
Variables collected included body mass, fat mass, fat-free mass, body water compartments and selected anthropometric indices.

An additional BIA measurement performed prior to study initiation (November 2025) was available for both athletes from their personal nutritionist. Due to differences in instrumentation and measurement protocols, these data were used only for descriptive purposes and not included in longitudinal comparisons.

3.7 Training Monitoring and Athlete's Diary

During the study period, participants were asked to complete a structured athlete diary designed to monitor training-related and lifestyle variables throughout the intervention (see Appendix C for the structure of the Athlete's Diary).

The diary included daily or weekly records of training sessions and training load, perceived fatigue and recovery status, GI symptoms, dietary adherence and supplementation intake and general wellbeing and health-related notes.

These records allowed continuous monitoring of the athletes during the study and provided essential contextual information for the interpretation of microbiota data, particularly in relation to training load, recovery dynamics, and lifestyle-related factors that may influence gut microbiota composition and functional potential.

3.8 Performance and Training Parameters

Performance-related data were collected through athlete self-report and sport monitoring platforms (e.g., Garmin, Coros, Strava), as specified in the project documentation (see Appendix A).

Available variables included estimated VO_2max derived from wearable devices, training load metrics, and race performance times. Due to variability in device use and data availability between participants, not all parameters were consistently recorded across the study period.

Given the observational and exploratory nature of the study, performance-related metrics were primarily used for descriptive purposes and for exploratory correlation analyses with microbiota data.

3.9 Statistical Analysis

Given the pilot nature of the study and the small sample size (n = 2), data analysis was primarily descriptive.

Microbiota composition was reported as relative abundance percentages at phylum, genus, and species level, while functional pathway outputs were analyzed using a descriptive approach.
Where appropriate, intra-individual comparisons were considered to explore potential longitudinal changes over the intervention period. However, as the follow-up microbiota assessment is planned and not yet completed at the time of writing, these comparisons will be performed in subsequent analyses.

Exploratory analyses were conducted to assess potential associations between microbiota variables and selected performance and body composition parameters. Due to the limited sample size, no formal inferential statistical conclusions were drawn, and any observed trends were interpreted with caution.

Data were organized and processed using spreadsheet software (Microsoft Excel), and, where applicable, additional statistical tools were used for exploratory purposes.

4. Results

4.1 Baseline characteristics, lifestyle and nutritional habits

Baseline demographic, clinical and lifestyle information for both athletes were collected through a structured anamnesis questionnaire prior to microbiota analysis.

The study included two male endurance athletes. Athlete A was a 29-year-old elite middle- and long-distance runner with a height of 174.5 cm and a body weight of 61.5 kg, corresponding to a body mass index (BMI) of 20.2 kg/m^2. Athlete B was a 23-year-old endurance runner with a height of 175 cm and a body weight of 60 kg, with a BMI of 19.6 kg/m^2.

Both athletes reported a natural full-term birth and exclusive breastfeeding during infancy. Early childhood was spent in urban environments. Athlete A reported occasional antibiotic use during the first three years of life due to influenza episodes, whereas Athlete B reported two antibiotic courses during early childhood.

Athlete A reported mild GI symptoms, including occasional meteorism, flatulence and episodes of abdominal tension. Bowel habits were characterized by daily evacuation, typically occurring in the morning before training after coffee consumption. Stool consistency was described as mixed, with a predominance of loose stools.

In contrast, Athlete B did not report chronic GI disorders. Bowel habits were described as regular, with one to two evacuations per day usually occurring after meals. Stool consistency was generally well-formed, although a temporary episode of looser stools was reported in November 2025 and subsequently resolved. Neither athlete reported previous diagnoses of GI disease.

Both athletes reported occasional oral symptoms, including aphthae and sporadic gingival bleeding. Athlete A also reported recurrent dental caries, whereas Athlete B reported occasional herpes simplex episodes. In both cases, a mild white coating of the tongue was described, possibly associated with dehydration.

Regarding extra-intestinal conditions, Athlete A reported two episodes of hematuria associated with dehydration, while Athlete B reported seasonal allergic rhinitis and mild asthma during springtime, as well as a congenital heart murmur without diagnosed cardiovascular disease. No hepatic, thyroid, dermatological or genitourinary diseases were reported.

Family history for Athlete A revealed several metabolic and GI conditions, including type 2 diabetes in both parents, celiac disease in the athlete's sister and Mediterranean anemia in the mother and sister. The athlete tested negative for anti-transglutaminase antibodies.

Athlete A reported previous episodes of gastroenteritis with vomiting and diarrhea during travel to Kenya in 2018 and food poisoning in Italy in 2020. Athlete B reported gastroenteritis with diarrhea during travel to Kenya in 2024 and one episode of COVID-19 infection.

No chronic diseases were reported at the time of the evaluation. Athlete A had a history of stress fractures of the femur, while Athlete B reported pubalgia at the time of the assessment.

Neither athlete reported ongoing pharmacological therapies, except for occasional use of ibuprofen for musculoskeletal pain. Athlete B also reported occasional use of fexofenadine during allergy periods.

Both athletes reported regular use of dietary supplements, although with markedly different patterns.

Athlete A followed a structured and performance-oriented supplementation regimen, including carbohydrate-based products (gels and maltodextrins), protein supplements, recovery formulations and ergogenic aids (e.g., creatine, nitrates and bicarbonate), in addition to micronutrient supplementation, particularly iron, vitamin C and magnesium.

In contrast, Athlete B reported a more heterogeneous and less structured supplementation pattern, including protein supplements and carbohydrate-based recovery products, as well as health-oriented compounds such as

multivitamins, lactoferrin, quercetin and propolis, together with joint-support supplements.

Overall, these supplementation practices frequently involved the use of processed formulations containing various excipients and additives, which may represent an additional environmental exposure potentially relevant to gut microbiota composition.

Following the initial assessment, a phytotherapeutic immune-support protocol was proposed to Athlete B; however, the athlete did not adhere to this recommendation.

Both athletes reported following an omnivorous diet characterized by a high carbohydrate intake typical of endurance athletes.

Athlete A reported frequent consumption of refined wheat pasta (approximately 12 times per week), whereas Athlete B reported pasta consumption approximately six times per week and rice intake three to four times per week. Bread and cereal-based products were frequently consumed by Athlete B, including homemade bread and multigrain products.

Fruit intake was approximately one portion per day for Athlete A and one to two portions per day for Athlete B. Vegetable consumption was low in both athletes, particularly in Athlete A, who reported vegetable intake only 2–3 times per week, while Athlete B reported approximately one portion per day, mainly consumed at dinner.

Protein sources included poultry, eggs and dairy products for both athletes. Athlete B reported higher overall meat consumption (5–6 times per week), whereas Athlete A reported moderate poultry and fish intake.

Extra virgin olive oil was the main dietary fat used by both athletes.

Daily fluid intake was reported as approximately 1.5 L per day for Athlete A and 2–2.5 L per day for Athlete B, increasing up to 3.5–4 L during training periods.

Both athletes reported moderate perceived stress levels.

Sleep duration was reported as 8.5–9 hours per night with excellent sleep quality for Athlete A and approximately 8 hours per night with good sleep quality for Athlete B.

Neither athlete reported smoking habits. Alcohol consumption was occasional and mainly limited to non-competitive periods.

Athlete B also reported living with a domestic dog and occasional use of chewing gum.

Table 1 – Baseline demographic, clinical and lifestyle characteristics of the two endurance athletes included in the study.

Variable	*Athlete A*	*Athlete B*
Sex	Male	Male
Age (years)	29	23
Height (cm)	174.5	175
Weight (kg)	61.5	60
BMI (kg/m²)	20.2	19.6
Sport discipline	Endurance running	Endurance running
Main competition distance	3000 m steeplechase, 1500 m, 5000 m, 10000 m, 10 km	10000 m, 10 km, half marathon
Birth mode	Natural delivery	Natural delivery
Breastfeeding	Yes	Yes
Early childhood environment	Urban	Urban
Antibiotic exposure (first 3 years)	Occasional	Two courses
GI symptoms	Occasional meteorism, flatulence, abdominal tension	No chronic GI symptoms
Bowel habits	Daily evacuation, mixed stools with loose tendency	1–2 evacuations/day, regular stools
Oral health issues	Aphthae, recurrent caries, occasional gingival bleeding	Occasional aphthae, herpes simplex, gingival bleeding
Extra-intestinal conditions	Episodes of hematuria linked to dehydration	Seasonal allergic rhinitis and mild asthma

Past infections	Gastroenteritis (Kenya 2018), food poisoning (2020)	Gastroenteritis (Kenya 2024), COVID-19
Musculoskeletal conditions	Stress fracture of femur	Pubalgia
Pharmacological therapy	Occasional ibuprofen	Occasional ibuprofen, fexofenadine
Dietary pattern	Omnivorous	Omnivorous
Pasta consumption	~12 times/week	~6 times/week
Fruit intake	~1 portion/day	1–2 portions/day
Vegetable intake	2–3 times/week	~1 portion/day
Fish consumption	1–3 times/week	<1 time/week
Egg consumption	3–4 times/week	3–4 times/week
Legume consumption	Occasional	2–3 times/week
Daily water intake	~1.5 L/day	2–2.5 L/day (up to 3.5–4 L training)
Coffee consumption	~3 cups/day	Occasional
Sleep duration	8.5–9 h/night	~8 h/night
Sleep quality	Excellent	Good
Smoking	No	No
Alcohol	Occasional	Occasional
Perceived stress	Moderate	Moderate

4.2 Body composition (BIA)

Athlete A

Body composition was assessed at baseline (30 January 2026) and follow-up (14 March 2026) using the same BIA-ACC device.

A slight increase in body weight was observed (61.5 to 62.5 kg), accompanied by a modest increase in fat mass (11.5 to 12.6 kg; 19% to 20%), while fat-free mass remained substantially stable (50.0 vs 49.9 kg).

Skeletal muscle mass showed minimal variation (19.0 to 19.1 kg), indicating preservation of the muscular compartment.

Total body water slightly decreased (35.2 to 34.9 L), while extracellular water percentage remained stable, suggesting no major alterations in hydration status.

Overall, body composition remained relatively stable over the intervention period, with minor variations within physiological ranges.

Athlete B

At baseline (30 January 2026), Athlete B presented a lean body composition with normal BMI (19.8 kg/m²) and balanced distribution between fat mass (20%) and fat-free mass (80%).

Total body water (55%) and extracellular water (42%) were within physiological ranges, indicating adequate hydration status.

Skeletal muscle mass was within the expected range for endurance athletes, although specific indices (e.g., SMI) suggested values close to the lower physiological threshold.

At follow-up (19 March 2026), Athlete B showed a slight increase in body weight (60.5 to 61.0 kg), associated with an increase in fat mass (11.8 to 12.6 kg; 20% to 21%), while fat-free mass slightly decreased (48.7 to 48.4 kg). Skeletal muscle mass showed minimal variation (18.2 to 18.1 kg), indicating relative stability of the muscular compartment despite the slight reduction in fat-free mass. Total body water decreased (33.0 to 32.6 L), with a corresponding reduction in percentage values (55% to 53%), while extracellular water percentage remained stable (42%), suggesting a mild reduction in hydration status rather than fluid redistribution. Overall, these changes indicate a slight shift toward increased adiposity and reduced hydration, with preservation of skeletal muscle mass within physiological ranges.

Pre-study body composition

Pre-study BIA assessments performed in November 2025 showed comparable body weight and overall body composition profiles in both athletes (e.g., 62.5 kg in Athlete A and 62.6 kg in Athlete B), supporting the stability of their anthropometric characteristics prior to the intervention.

However, these measurements were obtained using a different device and were therefore not included in the longitudinal analysis.

Table 2 – Body composition parameters assessed by BIA at baseline (T0) and follow-up (T1). Δ represents the absolute change between time points.

Variable	*A T0*	*A T1*	*Δ A*	*B T0*	*B T1*	*Δ B*
Weight (kg)	61.5	62.5	+ 1.0	60.5	61.0	+ 0.5
BMI (kg/m²)	20.3	20.6	+ 0.3	19.8	19.9	+ 0.1
Fat Mass (kg)	11.5	12.6	+ 1.1	11.8	12.6	+ 0.8
Fat Mass (%)	19	20	+ 1	20	21	+ 1
Fat-Free Mass (kg)	50.0	49.9	- 0.1	48.7	48.4	- 0.3
Skeletal Muscle (kg)	19.0	19.1	+ 0.1	18.2	18.1	- 0.1
Total Body Water (L)	35.2	34.9	- 0.3	33.0	32.6	- 0.4
Extra-Cellular Water (%)	43	43	0	42	42	0
HPA axis	3.4	3.6	+ 0.2	4.0	4.2	+ 0.2

4.3 Training characteristics

Training characteristics were obtained from the athletes' training logs recorded during the weeks preceding the microbiota assessment.

Athlete A followed a structured endurance running program characterized by a combination of aerobic conditioning, interval training, strength work and technical drills. Training was performed almost daily and frequently included two sessions per day.

Weekly training volume ranged approximately between 100–120 km per week. The training program was primarily based on aerobic endurance sessions, typically performed at low-to-moderate intensity, with paces generally around 4'00''/km to 4'10''/km during easy runs. These sessions commonly ranged from 30 minutes to 1 hour, and were periodically extended to longer runs of up to 20 km at moderate aerobic intensity.

High-intensity training sessions were regularly incorporated and consisted mainly of interval workouts performed on track. These included repeated efforts such as 10 × 1000 m with 1'30'' recovery (performed at approximately 2'48''–

2’50’’ per repetition), as well as mixed interval sets combining 1200 m, 1000 m and 800 m repetitions with short recovery intervals (around 1’30’’), typically performed at paces between approximately 2’50’’/km and 3’30’’/km depending on the distance.

Sprint drills and neuromuscular work were also included, such as short repetitions of 100–200 m and technical sessions involving hurdles, often combined with flexibility, coordination and mobility exercises.

In addition to running sessions, complementary training components were systematically integrated into the weekly program. These included strength training sessions, core stability work, and stretching routines, often performed either as separate sessions or in combination with low-intensity runs. Recovery-oriented sessions were also present and included easy runs and dedicated mobility work.

During specific training phases, Athlete A also performed heat training sessions, typically 2–3 times per week, conducted in controlled environments characterized by elevated temperature and humidity. These sessions were implemented as part of the overall training strategy and represented an additional physiological stressor aimed at enhancing thermoregulatory and endurance adaptations.

During the monitored period, Athlete A also participated in competitive events, including a 5 km race completed in 13:48 (2’47’’/km) and a 10 km road race completed in 29:11 (2’55’’/km). These competitions were integrated within the training program and contributed to overall training load and intensity distribution.

Overall, the training program combined high weekly running volumes with structured interval sessions, technical drills, complementary strength and mobility work, and occasional competitions, reflecting a comprehensive and periodized approach typical of competitive middle- and long-distance runners.

Athlete B initially presented a markedly reduced and unstructured training pattern compared to Athlete A, primarily due to the presence of chronic pubalgia and recurrent episodes of illness during the early phase of the monitoring period.

In particular, the athlete reported repeated URTIs, beginning with a febrile episode in late December, followed by persistent symptoms such as sore throat, cough and rhinitis that never fully resolved, and subsequent relapses, including a more acute episode shortly before the follow-up assessment.

During this phase, running activity was temporarily suspended and replaced by alternative low-impact modalities, including elliptical training and swimming, often performed within the same day, in order to maintain aerobic conditioning while minimizing mechanical stress.

Following recovery, which occurred around early March after physiotherapy and magnetotherapy, the athlete progressively resumed running and re-established a more structured training routine. The current training program was characterized by a weekly organization including double low-intensity sessions (8–10 km per session), strength training (mainly bodyweight and elastic strength exercises), interval sessions, medium-long runs (~16 km), and longer progressive or hilly sessions (16–20 km).

At full training load, weekly running volume is estimated at approximately 100–120 km, with most low-intensity sessions performed at an average pace of around 4’00’’/km. A typical training week includes double easy runs at the beginning of the week, strength training sessions, one high-intensity interval session, a medium-long aerobic run, and a long progressive or hilly session during the weekend.

Despite this progression, the training pattern remained partially influenced by the initial interruption, with a relatively recent transition toward structured training and potential limitations in long-term training continuity and cumulative load.

Overall, Athlete B’s training profile can be described as initially disrupted and subsequently reorganized, reflecting the combined impact of injury and recurrent infections on training consistency and potentially on physiological adaptation processes.

4.4 Dietary changes during the intervention period T0–T1

During the intervention period, dietary modifications were monitored qualitatively based on follow-up visits and self-reported information, as the completion of structured dietary records was inconsistent across participants.
Overall, the two athletes showed markedly different patterns of adherence to the proposed nutritional intervention.

Athlete A reported several relevant dietary modifications over the intervention period. In particular, an increased intake of fiber-rich foods was observed, including a higher consumption of legumes and a greater diversification of carbohydrate sources, with partial reduction in the reliance on refined wheat-based products such as pasta. Vegetable intake improved both in frequency and variety, with vegetables being regularly included in both main meals. In addition, hydration habits improved, with an increase in daily fluid intake compared to baseline. These changes suggest a partial but meaningful adherence to the proposed dietary strategy.

In contrast, Athlete B did not report substantial dietary modifications. The dietary pattern remained largely unchanged compared to baseline, characterized by a high intake of refined carbohydrate sources, limited consumption of vegetables and plant-based foods, and low dietary diversity. Adherence to the proposed nutritional intervention appeared minimal, with no consistent implementation of the recommended changes.

Due to the lack of quantitative dietary records, these observations should be interpreted as qualitative indicators of dietary behavior rather than precise measurements of nutrient intake. Nevertheless, the reported differences between the two athletes provide relevant contextual information for the interpretation of clinical and microbiota-related findings.

Table 3 – Qualitative overview of dietary patterns at baseline (T0) and during the intervention period (T1)

Variable	*A T0*	*A T1*	*B T0*	*B T1*
Refined carbohydrates	**High (frequent pasta intake)**	**Reduced, more diversified sources**	**High**	**Unchanged (high)**
Whole grains/ alternative carbs	**Limited**	**Increased (e.g., rice, barley)**	**Limited**	**Minimal / unchanged**
Vegetable intake	**Low frequency, low variety**	**Increased frequency and variety**	**Moderate (mainly dinner)**	**Unchanged, low variety**
Legumes	**Occasional**	**Frequent (almost daily)**	**Moderate**	**Unchanged**
Fruit intake	**~1 portion/day**	**Slight increase**	**1–2 portions/day**	**Unchanged**
Dietary diversity	**Low**	**Improved**	**Low–moderate**	**Unchanged (low)**
Hydration	**Suboptimal (~1.5 L/day)**	**Improved (~2–2.5 L/day)**	**Adequate**	**Slightly reduced**
Adherence to intervention	**–**	**Moderate**	**–**	**Low**

Data are presented as qualitative observations based on self-reported dietary habits and follow-up interviews.

4.5 Gut microbiota composition at baseline

Baseline gut microbiota composition was assessed through shotgun metagenomic sequencing of microbial DNA extracted from fecal samples. Unlike targeted 16S rRNA sequencing approaches, shotgun metagenomics allows a comprehensive characterization of the intestinal microbiome, enabling the identification of bacteria, fungi, DNA viruses and parasites at species level, as well as the assessment of the microbial functional potential.

The analysis was performed by Wellmicro (Bologna, Italy). In addition to taxonomic profiling, the test included the evaluation of microbial functional potential through the identification and quantification of specific microbial genes involved in metabolic pathways. This functional analysis was based on gene

abundance inferred from metagenomic sequencing rather than on metabolomic measurements.

Ecological diversity of the gut microbiota

Ecological diversity indices describing microbial richness and community structure are reported in Table 4.

Table 4 – Ecological diversity indices of the gut microbiota.

Ecological index	*Athlete A*	*Athlete B*	*Reference*
Shannon index	2.58	3.25	> 3.55
Observed species	144	151	> 120
Pielou evenness	0.51	0.64	0.68

Athlete B showed higher microbial diversity and evenness compared with Athlete A, while both samples presented a number of observed species above the reference threshold.

Bacterial composition

Bacteria represented the dominant component of the gut microbiota in both athletes.

The relative abundance of bacterial phyla is reported in Table 5.

Table 5 – Relative abundance of bacterial phyla.

Phylum	*Athlete A (%)*	*Athlete B (%)*	*Reference (%)*
Bacteroidetes	60.9	70.7	26.1 – 66.0
Firmicutes	34.1	24.5	24.2 – 57.3
Actinobacteria	2.9	1.9	1.7 – 17.2
Proteobacteria	1.1	0.8	0 – 2.7
Verrucomicrobia	0.0	1.0	0 – 4.8

In both athletes, the microbiota was dominated by members of the phylum Bacteroidetes, followed by Firmicutes, while Actinobacteria and Proteobacteria were detected at lower relative abundances.

To further characterize the bacterial community structure, the taxonomic composition was examined at progressively finer taxonomic levels, including bacterial families and selected species.

The relative abundance of selected bacterial families is reported in Table 6.

Table 6 – Relative abundance of selected bacterial families.

Family	*Athlete A (%)*	*Athlete B (%)*	*Reference (%)*
Prevotellaceae	55.7	47.1	0.0 – 40.1
Lachnospiraceae	16.2	14.4	12.9 – 42.1
Bacteroidaceae	3.7	15.8	6.6 – 44.7
Oscillospiraceae	12.1	6.3	3.0 – 15.5
Veillonellaceae	3.7	0.9	0 – 2.9
Bifidobacteriaceae	0.6	1.1	0 – 11.1

Prevotellaceae showed the highest relative abundance among the families reported in both athletes, followed by members of the Lachnospiraceae, Bacteroidaceae and Oscillospiraceae families. Additional families detected included Veillonellaceae and Bifidobacteriaceae.

Selective bacterial taxa identified at species and genus level are reported below in order to better characterize the microbial profiles of the two athletes, with a focus on functionally relevant microbial groups.

Overall, both microbiota profiles can be classified as *Prevotella*-associated enterotypes. Notably, species of the genus *Segatella* were previously classified within the genus *Prevotella* following recent taxonomic reclassification; therefore, they belong to the same phylogenetic and functional group.

In this context, Athlete A showed a strongly *Prevotella*-dominant configuration, characterized by a marked predominance of *Segatella copri* (previously *Prevotella copri*), whereas Athlete B presented a less polarized *Prevotella*-associated profile, with a higher relative abundance of *Segatella hominis* (previously *Prevotella hominis*) and a more balanced distribution of taxa.

Table 7 – Relative abundance of Prevotella-related taxa (*Segatella* spp.) in Athletes A and B.

Taxon	*Athlete A (%)*	*Athlete B (%)*	*Reference (%)*
Segatella copri	55.03	6.84	0 – 36.31
Segatella hominis	0.40	39.87	0 – 0.33

As shown in Table 7, the species-level analysis highlights the different ecological organization of the two microbiota profiles. Athlete A is characterized by the clear predominance of *Segatella copri*, whereas Athlete B exhibits a more evenly distributed configuration, with *Segatella hominis*, markedly exceeding reference values, as the most abundant taxon but without extreme dominance.

From a functional perspective, both athletes presented several bacterial taxa associated with SCFA production, particularly butyrate-producing bacteria within the Firmicutes phylum, including *Faecalibacterium* spp*.*, *Agathobacter rectalis* (formerly *Eubacterium rectale*), *Roseburia* spp*.* and *Anaerostipes hadrus*. For clarity, only selected butyrate-producing taxa were reported, given their central role in intestinal barrier integrity and host–microbiome interactions.

Table 8 – Butyrate-producing bacterial taxa identified in Athlete A and Athlete B.

Taxon	*Athlete A (%)*	*Athlete B (%)*	*Reference (%)*
Faecalibacterium prausnitzii	2.01	4.86	0.26 – 7.30
***Faecalibacterium* spp.**	9.94	5.75	0.26 – 8.36
Agathobacter rectalis	6.50	1.45	0.11 – 11
Roseburia intestinalis	1.41	0.95	0.06 – 2.92
Roseburia inulinivorans	0.26	1.01	0.02 – 1.66
Anaerostipes hadrus	0.17	0.34	0.16 – 3.81
Coprococcus eutactus	0.01	0.12	0 – 0.16

As reported in Table 8, these taxa were present in both athletes, although with different relative distributions. In Athlete A, SCFA-producing bacteria coexisted with a strong dominance of *Segatella copri*, suggesting a more specialized and less evenly distributed ecosystem. In contrast, Athlete B showed a more

balanced representation of these taxa, reflecting a more homogeneous microbial structure.

In addition, taxa potentially involved in lactate metabolism and propionate production were evaluated, with particular focus on members of the Veillonellaceae family and related microbial groups. These taxa are reported in Table 9 and include both lactate-producing bacteria (e.g., *Lactobacillus*, *Streptococcus*, *Bifidobacterium*) and lactate-utilizing or propionate-producing bacteria (e.g., Veillonellaceae-related taxa), supporting potential cross-feeding mechanisms within the gut microbiota.

Table 9 – Lactate-related and propionate-associated microbial taxa in Athletes A and B.

Taxon	*Athlete A (%)*	*Athlete B (%)*	*Reference (%)*
***Veillonella* spp.**	0	0.04	0.03 – 0.43
Veillonellaceae	3.7	0.9	0 – 2.9
Phascolarctobacterium faecium	0	1.31	0 – 1.60
***Dialister* spp.**	3.97	0.93	0.10 – 3.04
***Mitsuokella* spp.**	0	0.97	0.01 – 0.08
***Bifidobacterium* spp.**	0.63	1.16	0 – 8.14
***Lactobacillus* spp.**	0.03	0	0 – 0
***Streptococcus* spp.**	0.41	0.19	0.02 – 1.79

As shown in Table 9, *Veillonella* spp. were detected at negligible levels in either athlete. However, other members of the Veillonellaceae family, as well as taxa functionally associated with lactate production—such as *Lactobacillus* spp., *Streptococcus* spp. and *Bifidobacterium* spp.—were identified.

These microbial groups are known to contribute to lactate availability within the gut environment, which may serve as a substrate for propionate production through alternative metabolic pathways. Their presence suggests that, despite the low abundance of *Veillonella*, lactate–propionate cross-feeding mechanisms may still be supported by a broader microbial network.

Fungal component (mycobiome)

Fungal taxa belonging to the intestinal mycobiome were also detected. The relative abundances are reported in Table 10.

Table 10 – Relative abundance of fungal taxa.

Taxon	*Athlete A (%)*	*Athlete B (%)*
Aspergillus flavus	56.04	10.51
Aspergillus niger	12.64	2.83
Mucor velutinosus	6.59	85.45
Pichia kudriavzevii	24.73	0
Saccharomyces cerevisiae	0	1.21

Fungi represented a minor component of the intestinal microbial community in both samples.

DNA viruses and parasites

Metagenomic screening for DNA viruses and intestinal parasites was also performed. The analysis included a comprehensive panel of 23 parasites (protozoa and metazoa) relevant to human health, such as *Giardia*, *Toxoplasma*, *Blastocystis*, *Taenia*, *Filaria*, and *Entamoeba*, as well as a broad panel of over 700 DNA viruses.

No pathogenic human DNA viruses or intestinal parasites were detected in either athlete.

Functional potential of the microbiome

The functional potential of the microbiome was evaluated through the detection of microbial genes associated with metabolic pathways. The relative abundance of predicted metabolic functions is reported in Table 11.

Overall, the gut microbiota profiles of the two athletes showed distinct taxonomic compositions while sharing several bacterial taxa associated with SCFA production and carbohydrate metabolism.

Table 11 – Predicted metabolic functional potential of the gut microbiome.

Metabolic pathway	*Athlete A*	*Athlete B*
Acetate	+ 4	+ 1.9
Butyrate	- 1.9	- 2.4
Propionate	+ 4	+ 1.9
Succinate	- 1.1	+ 1.9
Lactate	- 0.7	- 0.2
Gamma-aminobutyric acid (GABA)	- 1.4	- 1.1
Serotonin	- 0.5	- 0.2
Histamine	+ 0.2	- 0.2
Indole	- 1.9	- 0.8
Indole-3-acetic acid (IAA)	- 0.5	- 0.2
Indole-3-propionic acid (IPA)	- 0.5	- 0.2
Tryptamine	- 0.3	- 0.2
Trimethylamine (TMA)	0	- 0.2
Putrescine	- 1.1	- 0.3
Cadaverine	- 1.1	- 0.3
Spermidine	+ 0.6	- 0.3
Polyphenols	- 0.1	- 0.2
B-group vitamins	- 0.6	- 0.5
Vitamin K2	- 0.5	- 0.5
Lipopolysaccharide (LPS)	0	0
Secondary bile acids	+ 0.4	+ 0.1
Ethanol	+ 0.3	0
Hydrogen sulfide	0	0
Methane	- 0.3	- 0.3
Fumarate	- 0.4	+ 1.9
Pyruvate	- 0.6	+ 2
β-glucuronidase (GUS)	+ 0.1	- 0.5
Sulfatase	- 1	- 0.6

5. Discussion

Although the intervention was initially guided primarily by clinical, dietary and lifestyle considerations, the microbiota results provided an additional interpretative framework for understanding the athletes' profiles and refining the rationale of the subsequent intervention strategy. For this reason, the discussion first addresses the microbiota findings and then contextualizes them within the broader nutritional and clinical framework.

5.1 Interpretation of gut microbiota results

The interpretation of the gut microbiota profiles was conducted in light of current evidence on the microbiome of athletes, with particular reference to the metagenomic study by Fontana et al. (2023). This study analyzed over 400 datasets comparing elite athletes, moderately active individuals, and sedentary subjects, identifying distinct taxonomic and functional signatures associated with different levels of physical activity.

This framework was used to contextualize the microbial composition observed in both athletes, allowing for a comparative evaluation of features potentially linked to athletic performance and training status.

5.1.1 Athlete A

High-level of physical activity is associated with specific microbial adaptations, characterized by an enrichment of taxa involved in the production of bioactive metabolites potentially relevant for host metabolism and exercise performance (Barton et al. 2018). In particular, athletes' microbiomes are often enriched in SCFA-producing bacteria, including *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and species belonging to the genera *Ruminococcus* and *Blautia*, which collectively represent a functional core microbiota associated with athletic status.

In this context, the gut microbiota of Athlete A showed a profile partially consistent with these findings, although characterized by specific features that deserve further consideration.

Overall microbial profile and ecological structure

The metagenomic analysis revealed a relatively high microbial richness (144 observed species), exceeding the reference threshold (>120 species). However, ecological diversity indices were markedly reduced (Shannon index 2.58; Pielou evenness 0.51), indicating an uneven distribution of taxa and the presence of a dominant microbial component.

This pattern suggests that the microbiota is rich in terms of species but ecologically unbalanced, with a strong dominance of specific taxa influencing community structure.

Prevotella-dominant configuration

The most distinctive feature of the microbiota profile was the marked predominance of *Segatella copri* (formerly *Prevotella copri*), which accounted for approximately 55% of the total bacterial community.

Prevotella-dominant microbiota configurations have been widely associated with carbohydrate-rich dietary patterns and have also been reported in endurance athletes (Fontana et al., 2023). Although this enterotype is traditionally linked to a high intake of plant-derived fibers, several studies have shown that it may also be associated with a high overall carbohydrate intake, including refined carbohydrates (Wu et al. 2011).

In the present case, despite the relatively low intake of vegetables, the high consumption of carbohydrate-rich foods typical of endurance training (e.g., pasta and carbohydrate supplements) may have contributed to the expansion of *S. copri*. It is also worth noting that Athlete A, although born and raised in Italy, has a Moroccan family background. Cultural and early-life dietary exposures associated with this background may have contributed to shaping long-term dietary habits and, potentially, gut microbiota configuration. However, this aspect was not specifically investigated and should be interpreted with caution.

The extremely high relative abundance of *Segatella copri* indicates a strongly Prevotella-dominant enterotype and likely contributes to the reduced ecological evenness observed in this sample. Similar configurations have been described

in non-Western populations characterized by distinct dietary patterns, particularly in rural African settings, highlighting the role of long-term dietary exposures in shaping gut microbiota structure (De Filippo et al. 2010).

Recent literature further supports the association between *Prevotella*-dominant microbiota and endurance training. Elite endurance athletes have been shown to exhibit a higher relative abundance of *Prevotella*, including *Prevotella copri*, compared to sedentary individuals, suggesting that *Prevotella*-enriched configurations may represent a microbiota signature associated with endurance training and high carbohydrate availability (Kulecka et al. 2020).

However, the functional implications of *Prevotella* dominance remain controversial. More recent evidence indicates that increased abundance of *Prevotella copri* may be associated with elevated inflammatory responses following exercise (Nieman et al. 2025). This suggests that, while *Prevotella*-dominant microbiota may reflect metabolic adaptation to endurance training, an excessive predominance of this taxon could also represent a potential critical aspect, particularly in relation to post-exercise inflammation.

In this context, the marked dominance of *Segatella copri* observed in Athlete A may be interpreted as a highly specialized microbial configuration potentially linked to endurance-related metabolic demands, but also as a condition that could contribute to an increased inflammatory susceptibility under conditions of physiological stress.

SCFA-producing bacteria

In addition to Prevotella dominance, the microbiota included several taxa known for their role in SCFA production, particularly butyrate-producing bacteria belonging to the Firmicutes phylum, such as *Agathobacter rectalis* (formerly *Eubacterium rectale*), *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Coprococcus* spp.

These microorganisms play a key role in maintaining intestinal homeostasis, contributing to epithelial integrity, immune modulation and energy metabolism. Their presence suggests that, despite the ecological imbalance, the microbiota

retains functional elements typically associated with a beneficial metabolic profile.

Notably, the microbiota of Athlete A also included bacterial species potentially involved in the regulation of exercise performance. In particular, *Agathobacter rectalis* (formerly *Eubacterium rectale*) and *Coprococcus eutactus* have been identified as microbial taxa capable of enhancing exercise capacity in experimental models. Recent evidence shows that mono-colonization with these species increases endurance performance, suggesting a causal role of specific gut microbes in physical activity regulation, potentially mediated through gut–brain axis mechanisms and dopaminergic signaling (Dohnalová et al. 2022), although translation to human physiology requires further investigation.

In this context, their presence may be consistent with a microbiota functionally oriented toward metabolic efficiency and endurance performance, in line with the highly specialized microbial configuration observed in this athlete.

Functional potential of the microbiome

The functional analysis indicated a generally well-preserved metabolic potential, with high scores in pathways related to intestinal barrier function, immune homeostasis and host–microbiome interaction axes, including the gut–muscle and gut–cardiovascular axes.

From a metabolic perspective, the microbiota showed an increased potential for acetate and propionate production, while butyrate production appeared slightly reduced. This pattern may reflect the compositional structure of the microbiota, characterized by *Prevotella* dominance, which is often associated with increased succinate production and, indirectly, propionate generation through microbial cross-feeding, rather than butyrate synthesis (Kovatcheva-Datchary and Arora 2013; De Filippis et al. 2016; Koh et al. 2016).

Microbial diversity and ecological imbalance

The reduced Shannon and Pielou indices can be primarily explained by the extreme dominance of *S. copri*. In ecological terms, this reflects a condition of

competitive exclusion, where a highly abundant taxon limits the expansion of other microbial groups.

Such configurations have been described in microbiota characterized by strong metabolic specialization, where specific taxa expand in response to the availability of preferred substrates. In endurance athletes, this may be related to the high and repeated intake of carbohydrates, which can promote the growth of efficient carbohydrate-fermenting bacteria.

Additionally, the relatively low dietary diversity, particularly the limited intake of vegetables, may have contributed to the reduced ecological balance of the microbiota.

Absence of *Veillonella*

Interestingly, no relevant abundance of *Veillonella* spp. was observed, despite its proposed role in endurance performance through lactate metabolism.

Interestingly, members of the Veillonellaceae family were present at relatively high levels, exceeding the reference range. This suggests that, although *Veillonella* itself was not detected, other taxa within the same family may contribute to overlapping metabolic functions.

Veillonella species utilize lactate as a substrate and convert it into propionate, a mechanism that has been proposed to support exercise performance (Scheiman et al. 2019). Their presence is typically associated with the availability of lactate and with lactate-producing bacteria such as *Lactobacillus*, *Streptococcus* and *Bifidobacterium*. In this case, the low abundance of these taxa may have limited lactate availability in the gut environment, thereby restricting *Veillonella* colonization. In parallel, the strong dominance of *Prevotella* may have contributed to shaping the microbial ecosystem toward alternative metabolic pathways.

Consistently, the functional analysis revealed a preserved or increased potential for propionate production, suggesting that this metabolic function may be supported by other microbial taxa, including *Prevotella*-related species and additional members of the Veillonellaceae family.

From a metabolic perspective, propionate represents a key SCFA that can be absorbed and transported to the liver, where it contributes to gluconeogenesis and energy homeostasis. In the context of endurance exercise, this pathway may support sustained energy availability, particularly during prolonged efforts. Therefore, despite the absence of *Veillonella*, the maintenance of propionate-producing capacity may still represent a relevant microbial contribution to host energy metabolism and exercise performance.

Overall, these findings highlight that similar functional outputs may be achieved through different microbial configurations, reinforcing the concept of functional redundancy within the gut microbiota.

Taken together, these findings suggest that, despite the presence of SCFA-producing taxa, the microbial ecosystem may be functionally imbalanced, with reduced butyrate production and altered cross-feeding dynamics.

Mycobiome, DNA viruses and parasites

The fungal component of Athlete A was characterized by a predominance of *Aspergillus* species, particularly *Aspergillus flavus* (56.04%) and *Aspergillus niger* (12.64%), along with a relevant presence of *Pichia kudriavzevii* (24.73%). Although fungi represented a minor fraction of the overall microbiota, the relative abundance of *Aspergillus* spp. is noteworthy. These fungi are ubiquitous environmental organisms commonly introduced through food or inhalation and are typically transient in the gastrointestinal tract. However, they may act as opportunistic pathogens or contribute to allergic and inflammatory responses under specific conditions, including high physiological stress, intense training load or altered mucosal barriers (Huffnagle and Noverr 2013; Underhill and Iliev 2014). In this case, their presence may be interpreted in light of Athlete A's clinical profile, characterized by mild gastrointestinal symptoms, recurrent oral issues (aphthae and caries), frequent consumption of refined carbohydrates and relatively low intake of plant-based foods, all of which may contribute to shaping a less stable microbial environment.

Overall interpretation

Overall, the gut microbiota of Athlete A can be described as metabolically active but ecologically unbalanced, characterized by high microbial richness, presence of SCFA-producing bacteria, preserved functional potential and strong dominance of *Prevotella copri*.

This profile suggests a microbiota adapted to high carbohydrate availability and endurance training demands, although at the expense of ecological diversity.

A shared feature observed in both athletes was a reduced butyrate-producing potential, indicating a functional shift of the microbiota toward acetate and propionate metabolism.

5.1.2 Athlete B

Ecological diversity and community structure

The gut microbiota of Athlete B was characterized by a total of 151 observed species, exceeding the reference threshold (>120), indicating a good level of microbial richness.

In contrast to Athlete A, ecological diversity indices were higher, with a Shannon index of 3.25 and a Pielou evenness of 0.64, although still slightly below reference values.

These findings suggest a more balanced microbial community compared with Athlete A, with a more even distribution of taxa and reduced dominance of a single species.

Prevotella-associated profile without extreme dominance

As previously observed for Athlete A, the microbiota of Athlete B also exhibited a Prevotella-associated configuration, characterized by the presence of Segatella-related taxa, particularly *Segatella hominis* (~40%) and, to a lesser extent, *Segatella copri* (~7%). As noted above, species of the genus *Segatella* were previously classified within the genus *Prevotella* and belong to the same phylogenetic and functional group.

However, in contrast to the strongly Prevotella-dominant profile described for Athlete A, Athlete B displayed a less polarized microbial structure, with a more balanced distribution of taxa and no extreme dominance of a single species. This suggests greater ecological evenness and potentially reduced competitive exclusion within the microbial community.

Rather than reiterating the dietary and functional associations of Prevotella described above, it is noteworthy that this more distributed configuration may reflect a different metabolic organization, in which fermentative activity is shared across multiple taxa rather than concentrated in a single dominant species. This could be associated with a more stable microbial ecosystem, potentially less prone to fluctuations under physiological stress.

Additionally, the lower relative abundance of *S. copri* compared to Athlete A may be relevant in light of recent evidence linking this species to post-exercise inflammatory responses (Nieman et al. 2025). While the presence of Prevotella-related taxa still suggests an adaptation to carbohydrate metabolism, the reduced dominance of *S. copri* and the coexistence of multiple taxa may indicate a less specialized but potentially more resilient microbial configuration.

In this context, the microbiota profile of Athlete B appears to differ not only in terms of taxonomic distribution but also in its potential functional implications. The clinical presentation of the athlete, characterized by recurrent infections and reduced physiological resilience, further suggests that microbiota composition should be interpreted within a broader multifactorial framework, including immune status, training load, and other host-related variables.

SCFA-producing bacteria and metabolic implications

As previously described for Athlete A, Athlete B also presented several taxa involved in short-chain fatty acid (SCFA) production, particularly within the Firmicutes phylum, including *Faecalibacterium prausnitzii*, *Agathobacter rectalis* (formerly *Eubacterium rectale*), *Roseburia intestinalis* and *Anaerostipes hadrus*.

However, in contrast to the more functionally oriented configuration observed in Athlete A, these taxa in Athlete B appeared more evenly distributed and not clearly dominant within the microbial ecosystem. This suggests a potentially

lower overall butyrate-producing capacity and a less pronounced contribution of SCFA metabolism at the community level.

Rather than indicating a highly specialized metabolic profile, this configuration may reflect a more heterogeneous functional organization, in which SCFA production is present but not a defining feature of the microbiota.

Athlete B also harbored microbial species previously associated with exercise performance, such as *Agathobacter rectalis* and *Coprococcus eutactus*. As discussed above, these taxa have been shown in experimental models to influence endurance capacity through gut–brain axis mechanisms (Dohnalová et al. 2022). However, in this case, their relative abundance and lack of dominance suggest a more limited or less coordinated functional impact compared to the profile observed in Athlete A.

Overall, despite the presence of potentially beneficial SCFA-producing and performance-associated taxa, the microbiota of Athlete B does not appear to exhibit the same degree of functional specialization. The clinical profile of the athlete, characterized by recurrent infections and reduced physiological resilience, further supports the interpretation that microbiota composition alone is insufficient to explain health and performance outcomes, which are likely influenced by multiple interacting host and environmental factors.

Propionate-related metabolism and Veillonellaceae

Although *Veillonella* spp. were detected only at negligible levels, the propionate production potential observed in this subject is likely supported by alternative microbial pathways. In particular, the presence of *Phascolarctobacterium faecium*, a well-known propionate producer, together with taxa such as *Dialister* and *Mitsuokella*, suggests an active succinate-to-propionate conversion pathway (Facchin et al. 2025). Moreover, the marked dominance of Prevotellaceae, which are primarily associated with succinate production, further supports the availability of metabolic precursors for propionate synthesis. These findings indicate that, in this athlete, propionate production is sustained by a distributed microbial network relying on cross-feeding mechanisms, rather than being driven by *Veillonella* abundance.

In addition, the low abundance of Actinobacteria, particularly *Bifidobacterium*, may have contributed to reduced lactate availability and altered microbial cross-feeding dynamics, further influencing the functional organization of the microbiota.

Functional potential of the microbiome

The functional profile of Athlete B showed a heterogeneous pattern, with moderately increased propionate and succinate production, relatively low butyrate production potential, reduced production of indole-derived metabolites and GABA and variable representation of pathways related to carbohydrate and amino acid metabolism.

Compared with Athlete A, the functional profile appeared less polarized and more evenly distributed across pathways, but not necessarily superior in terms of specific metabolite production.

This suggests a microbiota that is functionally versatile but less specialized.

Mycobiome, DNA viruses and parasites

The fungal component of Athlete B showed a marked predominance of *Mucor velutinosus* (85.45%), with lower relative abundance of *Aspergillus* species and minimal presence of *Saccharomyces cerevisiae*. Fungi represented a minor fraction of the microbiota and are likely reflective of environmental exposure rather than stable colonization. *Mucor* species are commonly found as environmental commensals in soil and food substrates and are generally considered non-pathogenic in healthy individuals; however, they can act as opportunistic pathogens, being associated with mucormycosis in conditions of impaired immunity or metabolic imbalance (Huffnagle and Noverr 2013; Underhill and Iliev 2014). In addition, recent clinical evidence suggests that *Mucor* spp. may also be involved in allergic respiratory conditions, including allergic bronchopulmonary mycosis, particularly in predisposed individuals (Zhang et al. 2022).

In this athlete, the high relative abundance of *Mucor velutinosus* may therefore be interpreted in light of both environmental exposure (e.g., diet or contact with

external sources such as domestic animals) and the clinical history of seasonal allergic rhinitis and mild asthma, suggesting a possible interaction between fungal exposure and respiratory immune responses. However, no direct pathogenic role can be inferred.

As in Athlete A, no pathogenic DNA viruses or parasites were detected.

Overall interpretation

The gut microbiota of Athlete B is characterized by a good microbial richness and a moderate ecological diversity, associated with a Prevotella-related profile without extreme dominance. The community showed a relatively balanced representation of SCFA-producing taxa and a functionally versatile profile, particularly in pathways related to carbohydrate metabolism.

Compared with Athlete A, this microbiota appears more evenly distributed across taxa and less dominated by a single species, suggesting a more balanced ecological structure.

However, this apparent ecological balance should be interpreted with caution. Despite these features, the athlete reported a higher susceptibility to recurrent infections and the presence of seasonal allergic rhinitis. These observations suggest that microbial diversity and compositional balance do not necessarily translate into optimal host resilience, which is likely influenced by multiple factors, including immune status, environmental exposures and psychological stress.

In this context, the marked predominance of *Mucor velutinosus* observed in the mycobiome may represent an additional factor of interest. Although generally considered an environmental commensal, *Mucor* spp. have been implicated not only in opportunistic infections but also, in rare cases, in allergic respiratory conditions such as allergic bronchopulmonary mycosis. This may be particularly relevant in individuals with underlying atopic conditions, suggesting a potential interaction between fungal exposure and respiratory immune responses. However, given the limited evidence and the observational nature of the present data, this finding should be interpreted cautiously.

Similarly to Athlete A, a reduced butyrate-producing potential was observed, reinforcing the presence of a shared functional pattern despite differences in microbial composition.

5.1.3 Comparative interpretation of gut microbiota profiles

The comparative analysis of the gut microbiota profiles of Athlete A and Athlete B revealed distinct ecological and functional configurations, despite similar endurance training regimens and broadly comparable dietary patterns.

As described above, Athlete A exhibited a low-evenness microbial ecosystem driven by the marked dominance of *Segatella copri*, whereas Athlete B displayed a more evenly distributed community structure without a single prevailing taxon. These findings suggest the presence of two alternative ecological strategies: a dominance-driven, specialized configuration in Athlete A and a more heterogeneous, distributed organization in Athlete B.

At the taxonomic level, both athletes shared a Prevotella-associated microbiota, although with different internal compositions, as previously outlined. The predominance of *S. copri* in Athlete A contrasted with the mixed Prevotella-related profile observed in Athlete B, indicating that species-level differences may contribute to distinct functional outputs.

Consistent with previous sections, SCFA-producing taxa were detected in both athletes, including *Faecalibacterium prausnitzii*, *Agathobacter rectalis* and *Roseburia intestinalis*. However, their ecological context differed, being embedded within a highly polarized structure in Athlete A and more evenly distributed in Athlete B. Notably, both athletes exhibited a reduced butyrate-producing potential, suggesting a shared functional shift toward acetate and propionate metabolism.

From a functional perspective, Athlete A showed a more polarized metabolic profile, whereas Athlete B exhibited a broader and less specialized functional distribution, as previously discussed. In both cases, propionate production appeared to rely on alternative microbial pathways independent of *Veillonella*, supporting the concept of functional redundancy within the gut microbiota.

This difference may reflect a more metabolically specialized microbiota in Athlete A, potentially better aligned with the energetic demands of endurance exercise, whereas the more heterogeneous profile observed in Athlete B may be associated with a less efficient metabolic organization. Although speculative, this could translate into a greater reliance on host physiological mechanisms to maintain energy homeostasis under similar training loads. However, this interpretation should be considered with caution, given the multifactorial nature of host–microbiome interactions.

Importantly, these microbiota configurations were not fully aligned with clinical outcomes. As previously reported, Athlete B—despite greater ecological balance—presented increased susceptibility to recurrent infections and allergic conditions, whereas Athlete A did not exhibit comparable clinical features. This discrepancy highlights the multifactorial nature of host–microbiome interactions, in which microbial composition represents only one of several contributing determinants.

Overall, these findings indicate that endurance athletes may harbor distinct but functionally plausible microbiota configurations, reflecting different adaptive strategies to similar physiological demands. As discussed above, these differences should also be interpreted in the context of individual dietary habits, supplementation patterns and environmental exposures, which likely contribute to shaping both microbial composition and metabolic potential.

5.2 Critical aspects and nutritional considerations

The analysis of dietary habits and lifestyle factors in both athletes highlighted several critical aspects that may have contributed to shaping gut microbiota composition and functional potential.

5.2.1 Athlete A

Low vegetable intake and insufficient fiber consumption

Athlete A reported a low and poorly diversified vegetable intake, limited to sporadic consumption (2–3 times per week), mainly in the form of ready-to-eat salads, with minimal inclusion of cooked vegetables.

This pattern likely results in an insufficient intake of functional fibers, micronutrients and polyphenols, which are essential for intestinal mucosal integrity, microbiota modulation and inflammatory regulation (De Filippis et al. 2016; Makki et al. 2018). The limited availability of diverse fermentable substrates may have contributed to the reduced ecological diversity observed in the microbiota.

Low dietary diversity and predominance of refined and processed carbohydrates

The diet appeared highly repetitive, with limited food rotation and a marked predominance of refined wheat-based products, particularly pasta consumed up to 12 times per week.

In addition to the high glycemic load, this pattern reflects a substantial reliance on industrially processed carbohydrate sources, which are typically characterized by reduced fiber content and altered nutritional profiles.

Dietary patterns rich in ultra-processed foods (UPFs) have been associated with alterations in gut microbiota composition, increased intestinal permeability, activation of pro-inflammatory pathways and low-grade systemic inflammation.

Importantly, these effects appear to be related more to the degree of processing than to the food category itself. In this context, the high intake of refined cereal products, combined with low fiber consumption, may have contributed to a reduced microbiota diversity and to an unstable intestinal environment (Heiman and Greenway 2016).

Unbalanced protein sources and high intake of industrial dairy products

Although total protein intake appeared quantitatively adequate, the dietary pattern was characterized by a predominance of dairy products and meat, with limited inclusion of fish and legumes. Notably, the frequent consumption of industrially processed dairy products (e.g., UHT milk, commercial yogurt and processed dairy formulations) may represent an additional factor influencing gut and systemic physiology.

Dietary patterns characterized by a high intake of ultra-processed foods (UPFs) have been associated with alterations in gut microbiota composition, increased intestinal permeability and activation of pro-inflammatory pathways, contributing to low-grade systemic inflammation. However, current evidence mainly supports an associative relationship rather than direct causality (Tristan Asensi et al. 2023). These effects are likely mediated by multiple factors, including high levels of refined carbohydrates and sugars, altered lipid profiles and the presence of food additives such as emulsifiers and preservatives, which have been shown to disrupt gut microbiota composition and impair intestinal barrier function (Chassaing et al. 2015; Zinöcker and Lindseth 2018).

Within this framework, the limited rotation of protein sources may further reduce the intake of nutrients relevant for inflammation control and gut barrier support.

Use of stimulants in the presence of altered bowel habits

The reported bowel pattern, characterized by evacuation following coffee intake and a tendency toward loose stools, suggests a regulation partially dependent on external stimuli rather than intrinsic intestinal balance. Notably, evacuative urgency was also reported following milk consumption, raising the possibility of an increased sensitivity to lactose or other milk components.

Overall, this pattern may reflect increased intestinal reactivity and could contribute to gastrointestinal instability, particularly during periods of elevated training load, when gut function is already exposed to significant physiological stress.

Inadequate hydration relative to training load

The reported fluid intake (~1.5 L/day) appears suboptimal considering the athlete’s training volume.

This may negatively affect GI function, mucosal hydration and systemic physiological balance, as suggested by clinical indicators such as dehydration-related hematuria and oral signs.

5.2.2 Athlete B

Limited variety of vegetable intake

As previously discussed for Athlete A, vegetable intake represents a critical determinant of microbiota modulation. In Athlete B, although consumption was daily, it was characterized by limited variety and concentration in a single meal. This pattern may restrict exposure to a diverse range of fermentable substrates and bioactive compounds, potentially limiting microbial adaptability.

Low dietary diversity and reliance on processed staple foods

Similarly to Athlete A, the diet showed limited variability and reliance on a narrow set of recurring foods. In this case, the issue appears less related to quantity and more to qualitative diversity, with insufficient inclusion of alternative plant-based sources and whole grains. This may constrain microbiota functional flexibility, particularly under chronic training stress.

Predominance of refined carbohydrates

Carbohydrate intake was largely derived from refined wheat-based products, with limited inclusion of alternative cereal sources. This pattern reduces the diversity of carbohydrate substrates and restricts the intake of different types of dietary fibers and prebiotic compounds typically found in other grains such as spelt, barley, maize and buckwheat.

As discussed above, low substrate diversity may limit microbiota metabolic flexibility and reduce ecosystem robustness, particularly when not balanced by adequate intake of fiber-rich and minimally processed foods.

Unbalanced protein sources and limited anti-inflammatory nutrients

Protein intake was mainly derived from meat, eggs and dairy products, with limited fish consumption. As outlined for Athlete A, low rotation of protein sources may reduce the intake of anti-inflammatory nutrients, particularly omega-3 fatty acids and plant-derived compounds, with potential implications for immune regulation and recovery.

5.3 Nutritional strategy and intervention rationale

Based on the identified dietary patterns and microbiota profiles, a targeted nutritional strategy was implemented in both athletes, with the aim of improving gut ecosystem stability, metabolic functionality and overall inflammatory balance.

The intervention was structured around four main pillars: reduction of UPFs and refined substrates, increase in plant food intake and seasonal dietary patterns, enhancement of dietary biodiversity and qualitative improvement of protein and fat sources.

Reduction of UPFs and refined substrates

A primary objective of the intervention was the reduction of UPFs, including refined wheat-based products (e.g., conventional pasta and bakery products) and industrial dairy formulations.

This choice was based on growing evidence indicating that diets high in UPFs are associated with alterations in gut microbiota composition, reduced microbial diversity, increased intestinal permeability, activation of pro-inflammatory pathways and low-grade systemic inflammation (Tristan Asensi et al. 2023).

Importantly, these effects appear to be related to the degree of food processing, rather than to the food category itself.

Therefore, both athletes were guided toward:

- replacing refined cereal products with minimally processed grains, including ancient wheat varieties, gluten-free cereals and pseudocereals,
- reducing consumption of industrial dairy products in favor of high-quality, minimally processed sources,
- limiting packaged and highly processed foods overall.

This shift aimed to reduce pro-inflammatory stimuli while improving the quality of substrates available to the gut microbiota.

Increase in plant food intake and seasonal dietary patterns

A second key component of the intervention was the increase in daily intake of plant-based foods, with a specific focus on consumption of at least two servings of vegetables per day, inclusion of seasonal and locally sourced produce and improvement in variety and rotation of plant foods.

Seasonal vegetables were prioritized to enhance micronutrient density, increase intake of polyphenols and fermentable fibers and support microbial diversity and metabolic cross-feeding (De Filippis et al. 2016; Makki et al. 2018).

Additionally, practical strategies were provided (e.g., meal preparation, cooking techniques, gradual introduction of legumes) to improve tolerability and adherence, particularly in subjects with GI sensitivity.

Enhancement of dietary biodiversity

Both athletes were encouraged to increase dietary diversity, a key determinant of gut microbiota richness and resilience (Heiman and Greenway 2016).

This included rotation of carbohydrate sources (cereals, pseudocereals, tubers), diversification of protein sources (legumes, fish, eggs, meat, high-quality dairy) and introduction of previously underrepresented foods (e.g., legumes, alternative grains).

Greater dietary diversity provides a wider range of substrates for microbial metabolism, promoting production of SCFAs, functional redundancy within the microbiota and increased ecosystem stability under physiological stress (Makki et al. 2018).

Qualitative improvement of protein and fat sources

Particular attention was given to the quality of protein and fat sources, with the aim of improving inflammatory balance and supporting gut barrier function. Recommendations included increasing intake of fresh fish (e.g., sardines, mackerel, salmon) and reducing reliance on processed or preserved products, improving the quality of meat (e.g., grass-fed, local sources) and selecting minimally processed dairy products.

From a lipid perspective, the intervention emphasized the use of extra virgin olive oil as the primary fat source, due to its high content of monounsaturated fatty acids and bioactive compounds such as polyphenols, which have been associated with anti-inflammatory effects and beneficial modulation of gut microbiota composition.

In parallel, greater intake of omega-3 fatty acids was encouraged through regular consumption of fatty fish and, where applicable, plant-based sources such as flaxseeds and walnuts. These lipids play a key role in regulating inflammatory pathways, modulating immune responses and supporting intestinal barrier integrity.

Overall, these modifications aimed to improve the fatty acid profile of the diet, promoting a more favorable balance between pro- and anti-inflammatory signals.

Individual considerations

Although the general principles of the intervention were shared, specific adjustments were made based on individual characteristics.

In Athlete B, particular attention was given to the allergic background (grass pollen allergy) and to potential food cross-reactivity phenomena. In subjects sensitized to airborne allergens, such as grass pollen, immune responses may occur against structurally homologous proteins present in plant-derived foods, a condition commonly referred to as pollen–food allergy syndrome or oral allergy syndrome (PFAS/OAS) (Kato et al. 2025; Rousou et al. 2025). These reactions are mediated by IgE cross-recognition of homologous proteins (e.g., PR-10, profilins, lipid transfer proteins), leading to localized or systemic allergic responses.

In this context, dietary recommendations included the modulation of potentially cross-reactive foods, particularly during periods of increased allergic symptomatology.

In addition, attention was given to the intake of histamine-rich and histamine-liberating foods. Although the role of dietary histamine in allergic conditions

remains not fully established, some evidence suggests that reducing histamine intake may help modulate symptom severity.

In patients with allergic rhinitis, the exclusion of histamine-rich and histamine-liberating foods has been associated with a significant improvement in clinical symptoms compared to standard treatment alone, supporting a potential role of dietary histamine modulation during symptomatic phases (Srivastava and Kaplan 2021).

From a mechanistic perspective, histamine represents a key mediator of allergic responses, contributing to vasodilation, mucus production and nasal symptoms (Maintz and Novak 2007; Comas-Basté et al. 2020). Therefore, reducing exogenous histamine load during periods of increased endogenous release (e.g., pollen exposure) may help limit the overall histamine burden in susceptible individuals.

In addition, emerging evidence suggests that the gut microbiota may contribute to histamine homeostasis, as certain bacterial taxa are capable of producing or degrading histamine, potentially influencing host inflammatory and immune responses (Frei et al. 2013; Barcik et al. 2016). This highlights a possible interaction between diet, microbiota composition and histamine-related symptomatology.

More broadly, the gut microbiota plays a key role in the regulation of immune responses relevant to allergic diseases. In particular, microbial-derived metabolites such as SCFAs have been shown to modulate immune function by promoting Treg differentiation and reducing allergic inflammation (Tan et al. 2014; Trompette et al. 2014).

In this context, dietary patterns that support SCFA production through increased intake of fiber-rich and minimally processed foods may contribute not only to gut microbiota stability but also to improved immune tolerance, potentially influencing susceptibility to allergic symptoms.

5.4 Follow-up outcomes and targeted supplementation strategy

The interpretation of follow-up findings should be considered in continuity with baseline microbiota profiles and in light of both the intervention strategy and the level of adherence.

5.4.1 Athlete A

Follow-up outcomes

At follow-up, Athlete A reported a body weight of 62.5 kg and a relevant improvement in GI symptoms and exercise tolerance. In particular, the athlete no longer experienced the need for bowel evacuation during running, a symptom previously reported. Prior to the initial assessment, the athlete frequently reported the need to interrupt training sessions due to urgent bowel movements. Following the dietary modifications, particularly the elimination of milk, bowel evacuation is now consistently occurring before training or competition, with no further interruptions during running.

These improvements occurred in parallel with the dietary modifications implemented during the intervention, including increased intake of fiber-rich foods, greater diversification of carbohydrate sources, and improved vegetable consumption. Hydration status also improved compared to baseline.

From a gut microbiota perspective, the increased consumption of fiber-rich foods may have contributed to a more favorable intestinal environment by providing fermentable substrates for microbial metabolism and SCFA production, which are associated with improved intestinal barrier function and reduced inflammation (Makki et al. 2018).

Improvements in recovery capacity were also reported, although these may have been partially influenced by additional recovery strategies (e.g., cryotherapy and heat application).

An additional relevant observation concerns race-day nutritional strategies and their potential impact on GI tolerance and performance. During the 10 km race performed on March 15, Athlete A reported the use of a carbohydrate gel that differed from his usual supplementation routine (previous sponsor product). The

gel was consumed early in the race (approximately at km 2), rather than prior to the start as typically practiced, and was later identified as expired.

Following ingestion, the athlete experienced upper GI discomfort characterized by reflux symptoms, which became particularly evident in the final phase of the race. At approximately km 9, despite the intention to increase pace, the presence of these symptoms required a slight reduction in intensity.

The athlete completed the race in 29:11, finishing in fourth position, just 1 second behind third place and 2 seconds behind second place. Although this performance remains competitive, it is plausible that suboptimal in-race nutritional management may have limited the ability to fully express performance capacity. The athlete's personal best at the time was 28:37, suggesting that a faster performance might have been achievable under optimal GI conditions.

From a gut microbiota perspective, this episode may reflect an acute perturbation of the intestinal environment induced by the ingestion of an unfamiliar and potentially poorly tolerated product. In athletes characterized by a microbiota adapted to habitual dietary and supplementation patterns, the introduction of novel substrates—especially under exercise-induced stress conditions—may transiently alter GI function, potentially affecting intestinal permeability, fermentation dynamics and symptom perception. This observation is consistent with the concept that gut microbiota–host interactions are highly sensitive to both chronic dietary patterns and acute nutritional exposures, particularly during endurance exercise.

This observation further supports the importance of individualized and well-tested nutritional strategies during competition, particularly in athletes with previous GI sensitivity, in whom gut microbiota–host interactions may be especially sensitive to acute nutritional exposures.

Adherence to dietary and supplementation intervention

Athlete A demonstrated a moderate level of adherence to the proposed intervention. Based on self-reported information, the athlete increased the intake of fiber-rich foods and partially reduced the consumption of refined carbohydrate sources. However, adherence could not be quantitatively

assessed due to the lack of completion of the dietary and training diary. This limitation should be taken into account when interpreting the observed clinical improvements.

Rationale of the intervention strategy

The nutritional and supplementation strategy for Athlete A was designed according to a targeted rationale based on microbiota findings and clinical presentation, with the primary objective of increasing beneficial taxa—particularly *Bifidobacterium* spp. and Firmicutes—and supporting intestinal barrier integrity.

This approach was supported by baseline microbiota findings, which indicated a relatively low abundance of Actinobacteria, particularly *Bifidobacterium* spp., together with a reduced butyrate-producing potential. These features suggested both a compositional and functional imbalance of the microbial ecosystem, with potential implications for intestinal barrier integrity and inflammatory regulation.

Accordingly, the intervention was aimed at enhancing short-chain fatty acid production—particularly butyrate—and restoring beneficial microbial taxa.

Dietary intervention

The dietary approach focused on increasing the intake of fiber-rich and minimally processed foods, particularly legumes and whole grains, in order to provide fermentable substrates for gut microbiota. This strategy aimed to enhance SCFA production, especially butyrate, which appeared functionally reduced at baseline and plays a key role in maintaining intestinal barrier integrity and regulating inflammation (Tan et al. 2014; Makki et al. 2018).

In addition, the inclusion of prebiotic compounds such as fructo-oligosaccharides (FOS), inulin, and galacto-oligosaccharides (GOS) was intended to selectively stimulate beneficial microbial taxa, including *Bifidobacterium* spp., which were underrepresented at baseline (Gibson et al. 2017).

Phase I: Stabilization and metabolic support

The first phase consisted of a synbiotic approach based on the alternate administration of two multi-strain probiotic formulations combined with prebiotic substrates. Specifically, the protocol included a formulation containing fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) associated with multiple probiotic strains, including *Lactobacillus salivarius* W57, *L. casei* W56, *L. acidophilus* W22, *L. rhamnosus* W71, *L. plantarum* W62, *Lactococcus lactis* W58 and *Enterococcus faecium* W54. This was alternated with a second formulation containing *Lactobacillus plantarum* LPB22, *L. acidophilus* LA3, *L. casei* LC18, *L. rhamnosus* LRH11, *L. fermentum* LF350 and *L. reuteri* LR07, combined with inulin as a prebiotic substrate.

The two formulations were administered on alternate days to increase strain diversity and expand the range of fermentable substrates available to the microbiota. This approach was designed to promote microbial stabilization, enhance colonization resistance and support metabolic activity through cross-feeding mechanisms.

Synbiotic interventions have been shown to modulate gut microbiota composition, enhance short-chain fatty acid production and support immune function (Hill et al. 2014; Sanders et al. 2019).

Phase II: Diversification and functional reinforcement

The second phase involved the administration of a broader-spectrum multi-strain formulation, including prebiotic inulin and a diverse consortium of bacterial species: *Bifidobacterium longum* DSM 25174, *B. bifidum* DSM 25565, *B. breve* DSM 25173, *Lactobacillus gasseri* LMG-P-29638, *L. casei* DSM 25569, *L. rhamnosus* DSM 25568, *Streptococcus thermophilus* DSM 26721, *Enterococcus faecium* LMG S-28935, and the spore-forming strain *Bacillus subtilis* BSM12.

The inclusion of a wider range of taxa, together with a spore-forming species, was intended to enhance microbial diversity and support ecosystem resilience, particularly under conditions of physiological stress. This second phase followed a stepwise strategy, transitioning from initial stabilization toward diversification and functional reinforcement of the gut microbiota.

Taken together, the intervention was designed as a coordinated and mechanism-driven approach targeting both microbial composition and host–microbiome interactions.

Interpretation of follow-up findings

The clinical improvements observed at follow-up, particularly the resolution of exercise-induced bowel urgency and the reported enhancement in recovery capacity, are consistent with the proposed intervention mechanisms.

The increase in dietary fiber intake, combined with targeted probiotic supplementation, may have contributed to improved microbial metabolic activity, enhanced SCFA production and better intestinal barrier function.

These findings are consistent with a partial restoration of microbial metabolic activity, particularly in relation to short-chain fatty acid production, although this remains to be confirmed by follow-up microbiota analysis.

Although the absence of quantitative adherence data limits the strength of causal inference, the coherence between the implemented changes and the observed outcomes suggests a positive interaction between dietary modulation, microbiota dynamics and host physiological response.

Additional considerations – ergogenic supplementation

In contrast to microbiota-targeted interventions, beta-alanine supplementation was introduced at the athlete's request, likely in alignment with performance-oriented strategies. Beta-alanine is a well-established ergogenic aid known to enhance exercise capacity through its role in carnosine synthesis and buffering of intramuscular acidity (Saunders et al. 2017).

However, it was not part of the microbiota-focused intervention and is therefore not expected to have directly influenced gut microbial composition. Current evidence suggests that most ergogenic supplements exert limited or indirect effects on the gut microbiota, with the strongest microbiota-modulating effects being associated primarily with dietary patterns, fiber intake and probiotic or prebiotic interventions (Katsimichas et al. 2025).

Accordingly, the inclusion of beta-alanine in this context should be interpreted as a performance-oriented intervention rather than a factor contributing to the observed microbial profiles.

5.4.2 Athlete B

Follow-up outcomes

At follow-up, Athlete B reported a body weight of approximately 61.0 kg, with a slight increase compared to baseline, mainly attributable to an increase in fat mass, while muscle mass remained substantially stable. A mild reduction in total body water was also observed, potentially reflecting suboptimal hydration status.

From a clinical perspective, the athlete showed complete resolution of pubalgia and a progressive return to regular training. However, recurrent upper respiratory tract symptoms persisted throughout the monitoring period, characterized by relapsing episodes following an initial febrile event. A further acute episode occurred shortly before follow-up, requiring short-term use of non-steroidal anti-inflammatory drugs (NSAIDs).

GI function was reported as stable, with regular bowel habits and no major symptoms.

Adherence to dietary and supplementation intervention

Adherence to the proposed intervention was overall low. No substantial dietary modifications were reported, and the athlete did not follow the recommended supplementation protocol, continuing instead with self-managed supplementation. The completion of the dietary and training diary was also partial and inconsistent, limiting the possibility of accurately quantifying adherence and interpreting follow-up outcomes.

Rationale of the intervention strategy

The supplementation strategy proposed for Athlete B was structured according to a targeted and stepwise rationale based on both microbiota findings and clinical presentation.

As part of the foundational intervention, vitamin D supplementation (in combination with vitamin K2) was initiated from Phase I and maintained throughout the intervention period. This choice was based on both laboratory findings and clinical presentation. The athlete showed suboptimal and progressively declining serum vitamin D levels over time, with values approaching insufficiency at the most recent assessment, measured at 32.5 ng/mL on October 31, 2025. In addition, blood test results obtained on October 31, 2025 showed elevated bilirubin levels, including total bilirubin (1.98 mg/dL; optimal <1.20 mg/dL), direct bilirubin (0.66 mg/dL; optimal <0.30 mg/dL) and indirect bilirubin (1.32 mg/dL; optimal <0.90 mg/dL). These findings suggested a potential alteration in hepatic function or bilirubin metabolism, further supporting the rationale for including interventions aimed at supporting liver function and detoxification pathways.

Vitamin D plays a key role in immune regulation, contributing to both innate and adaptive immune responses and being associated with a reduced risk of respiratory infections (Martineau et al. 2017). Its inclusion was therefore aimed at improving immune resilience, particularly in light of the athlete's history of recurrent URTIs.

In addition, vitamin D has been suggested to influence gut microbiota composition and intestinal barrier function, indicating a potential indirect role in host–microbiome interactions.

Phase I: Modulation of fungal component and hepatic support

This phase was informed by baseline microbiota findings, characterized by a marked predominance of *Mucor* and the presence of *Aspergillus* species, as well as by biochemical findings, particularly elevated bilirubin levels, which suggested the need for targeted support of hepatic function.

The intervention included a formulation containing N-acetylcysteine (NAC), silymarin (from *Silybum marianum*), S-adenosyl-methionine (SAMe), *Fumaria officinalis* extract, zinc and a plant-derived B-vitamin complex. These compounds were selected to support hepatic detoxification pathways and modulate oxidative stress.

NAC, as a precursor of glutathione, contributes to redox balance and has been shown to interfere with microbial biofilm formation, potentially enhancing microbial ecosystem resilience. Silymarin and SAMe are known for their hepatoprotective and metabolic regulatory roles. Although this approach does not represent a direct antifungal strategy, modulation of host detoxification pathways and oxidative status may indirectly contribute to rebalancing the gut microenvironment.

Alterations in the gut mycobiome have been associated with microbial imbalance and inflammatory responses, and their modulation may contribute to restoring ecological stability (Huffnagle and Noverr 2013; Underhill and Iliev 2014).

Phase II: Microbiota modulation and immune support

The second phase focused on restoring bacterial balance and supporting immune function. Based on microbiota analysis (reduced Firmicutes, low abundance of *Bifidobacterium* spp. and reduced butyrate-producing potential), a combined synbiotic and micronutrient-based approach was implemented.

The intervention included a multi-strain probiotic formulation containing *Bifidobacterium longum, B. infantis, B. bifidum, Lactobacillus paracasei, L. plantarum* and *L. rhamnosus*, combined with fructo-oligosaccharides (FOS) to selectively stimulate beneficial taxa.

In parallel, an antioxidant and immunomodulatory formulation was introduced, providing lysine, polyphenols (including anthocyanins from *Vaccinium myrtillus* and grape-derived compounds), vitamin C (as magnesium ascorbate) and trace elements (zinc, manganese, copper and selenium).

Lysine has been associated with the modulation of herpes simplex virus reactivation, while polyphenols exert anti-inflammatory and microbiota-modulating effects (Del Rio et al. 2013). This phase was therefore designed to act on both microbial composition and host immune response.

Phase III: Intestinal barrier support

The final phase aimed at reinforcing intestinal barrier integrity through a formulation containing palmitoylethanolamide (PEA), glutamine, butyric acid and quercetin.

These compounds are known to support epithelial function, modulate inflammatory pathways and reduce intestinal permeability. Butyrate plays a central role in maintaining gut barrier integrity and immune homeostasis, while glutamine serves as a primary fuel for enterocytes. PEA and quercetin contribute to immune modulation and control of low-grade inflammation (Tan et al. 2014).

Taken together, these interventions were designed as a coordinated strategy targeting microbial composition, immune regulation and host–microbiome interactions.

Interpretation of follow-up findings

Despite the presence of a structured and mechanistically grounded intervention strategy, the lack of adherence represents the main factor limiting the interpretation of outcomes in this athlete.

The persistence of recurrent infections, together with the absence of meaningful dietary changes, suggests that the proposed intervention was not effectively implemented. As a result, it is not possible to evaluate the potential impact of microbiota-targeted and immune-support strategies.

More broadly, this case highlights how microbiota composition alone does not determine clinical outcomes, and how behavioral factors—particularly adherence to dietary and supplementation protocols—play a central role in shaping both host physiology and microbiota dynamics.

5.5 Adherence to the intervention protocol

Adherence to the proposed dietary and supplementation interventions represents a critical factor in the interpretation of both clinical and microbiota-related outcomes. Several methodological limitations emerged during the study, potentially affecting data reliability.

The timing of microbiota sampling was not fully standardized between subjects. In one case, sample collection was delayed by approximately 10 days, and in both cases processing was not immediate due to procedural constraints related to online registration. Consequently, analysis occurred up to 15 days after collection, introducing a potential source of variability given the dynamic nature of the gut microbiota.

Adherence to the intervention also differed substantially between the two athletes and was assessed primarily through qualitative self-report. Athlete A demonstrated partial but meaningful compliance, although the absence of a completed dietary and training diary prevented a quantitative evaluation. In contrast, Athlete B showed minimal adherence to both dietary and supplementation protocols, with incomplete and inconsistent reporting.

Taken together, these factors represent a major limitation of the study, reducing the strength of causal inference and highlighting the importance of compliance and standardized procedures in microbiota-based interventions. These findings further emphasize that behavioral factors and adherence to lifestyle modifications are key determinants of both clinical outcomes and gut microbiota dynamics.

6. Conclusions

The present longitudinal pilot study was designed toinvestigate the composition and functional potential of the gut microbiota in competitive middle- and long-distance runners, and to explore early adaptations following a personalized nutritional and nutraceutical strategy. By integrating metagenomic profiling with clinical, nutritional and training-related data, this work provides a preliminary insight into the complex interplay between gut microbiota and physiological adaptation in endurance athletes within a real-world setting.

The results highlighted the presence of distinct microbiota configurations between the two athletes, despite similar training modalities. In both cases, microbial profiles were characterized by taxa associated with carbohydrate metabolism and SCFA production, although with different ecological structures and degrees of specialization. These observations support the concept that multiple microbiota configurations may coexist within athletic populations, reflecting individual variability in diet, training load, lifestyle and host-related factors.

This project was developed in collaboration with ASD Atletica Reggio, a well-established athletics association based in Reggio Emilia, counting approximately 1200 members, and representing the sporting context of the athletes involved in this study. This collaboration constitutes a key strength of the project, as it allows the investigation to be embedded in a real-world competitive environment and provides a solid foundation for future expansion of the study population.

Despite the exploratory nature of the study, one of the most relevant aspects emerging from this work concerns the critical role of adherence to the intervention protocol. Adherence was variable and, in one case, markedly limited, representing a major constraint in the interpretation of results and in the evaluation of the intervention effectiveness. This finding underscores how, in competitive athletes, organizational demands, training schedules, travel, and individual habits may significantly influence the feasibility of implementing nutritional strategies. Beyond the scientific rationale, the sustainability and real-

world applicability of an intervention therefore represent fundamental determinants of its success.

This study should be interpreted as a preliminary phase within a broader research pathway. A follow-up microbiota assessment is planned to evaluate longitudinal changes following the intervention, taking into account the constraints imposed by the athletes' competitive calendar. Future developments will focus on systematically investigating whether targeted nutritional strategies can effectively modulate gut microbiota composition and functional potential, and how such modulation may influence metabolic adaptation, gastrointestinal tolerance, immune resilience, and stress regulation in endurance athletes.

Further research will also explore the combined role of dietary strategies, lifestyle factors, and microbiota-targeted interventions within an integrated and personalized framework. In particular, expanding the study to amateur athletes—who represent a larger and less studied population—may enhance both the external validity and the practical applicability of the findings, supporting the development of scalable and sustainable interventions.

In conclusion, this pilot study contributes to the growing body of evidence supporting the gut microbiota as a potential modulator of physiological adaptation in sport. Importantly, it highlights the need to integrate biological, behavioral, and contextual factors in the design of future interventions, emphasizing that the effectiveness of microbiota-targeted strategies depends not only on their scientific foundation, but also on their feasibility and adherence in real-life settings.

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Appendices

Appendix A - Enrollment and Informed Consent Form

Titolo del progetto: Microbiota in Atletica di Endurance
Ente promotore: Dott.ssa Florencia Ceppa
Responsabile scientifico: Dott.ssa Florencia Ceppa

Premessa

Il presente documento ha lo scopo di informare il/la partecipante sulle finalità, modalità e condizioni di partecipazione al progetto di ricerca sopra indicato e di raccoglierne il consenso libero e informato.

Il progetto di ricerca ha una durata complessiva di **16 settimane** e si propone di analizzare la relazione tra pratica sportiva di endurance e composizione del microbiota intestinale.

Durata e impegno richiesto

La partecipazione al progetto prevede l'adesione alle procedure e alle indicazioni fornite dalla dott.ssa Florencia Ceppa per **l'intera durata dello studio (16 settimane)**.

Il/la partecipante si impegna a:

- attenersi alle indicazioni operative e metodologiche previste dal progetto di ricerca;
- comunicare tempestivamente eventuali difficoltà o impedimenti alla corretta adesione al progetto.

Nel caso in cui il/la partecipante, per **motivi personali, etici, religiosi o culturali** (ad esempio periodi di digiuno religioso) non possa attenersi alle indicazioni previste dal protocollo, è richiesto di **comunicarlo anticipatamente ai responsabili dello studio**, al fine di consentire una valutazione della compatibilità con il progetto di ricerca.

Criteri di inclusione

Possono partecipare al progetto soggetti che soddisfino tutti i seguenti criteri:

- atleti praticanti sport a prevalenza aerobica, quali:
    - mezzofondo;
    - fondo;
    - maratona;

    - altre discipline di endurance.
- età compresa tra 20 e 45 anni;
- assenza di condizioni cliniche rilevanti o patologie diagnosticate che possano interferire con lo studio;
- stato di buona salute generale;
- assenza di assunzione di antibiotici, probiotici, antiacidi negli ultimi 3 mesi e si invita a comunicare in fase di reclutamento tutti i farmaci ed integratori o alimenti funzionali (es. barrette, gel e altri preparati per lo sport) alla dott.ssa Florencia Ceppa per una valutazione.
- disponibilità a partecipare allo studio per l'intera durata prevista.

Volontarietà della partecipazione

La partecipazione allo studio è **volontaria** e prevede il versamento di un **contributo economico**, finalizzato alla copertura dei costi organizzativi e di ricerca, secondo le modalità che verranno comunicate dalla dott.ssa Florencia Ceppa.

Il/la partecipante può **ritirare il proprio consenso in qualsiasi momento**, senza obbligo di fornire spiegazioni e senza che ciò comporti conseguenze di natura disciplinare o personale.
Resta inteso che, in caso di recesso anticipato, **il contributo economico eventualmente versato non sarà rimborsato**, salvo diversa indicazione dalla dott.ssa Florencia Ceppa.

Trattamento dei dati

I dati raccolti durante lo studio saranno trattati in forma anonima e aggregata, nel rispetto
della normativa vigente in materia di protezione dei dati personali
(Regolamento UE 2016/679
– GDPR). I risultati potranno essere utilizzati esclusivamente per finalità scientifiche e di
ricerca.

CONSENSO INFORMATO E INFORMATIVA PRIVACY

Prestazione sanitaria nutrizionale e utilizzo dei dati in forma anonima

Progetto "Microbiota in Atletica di Endurance – Atletica Reggio ASD"

1. Titolare del trattamento e responsabile sanitario

Dott.ssa Florencia Andrea Ceppa, Biologa Nutrizionista, Farmacista, PhD
Iscrizione Ordine dei Biologi dell'Emilia-Romagna e delle Marche n. ERM_A04604
Studio professionale: Via Premuda 30/30 – 42124 Reggio Emilia
P. IVA 02576130229 – C.F. CPPFRN86C70Z600Y
Email: florencia@byotics.it – Tel: +39 351 604 3188

2. Riferimenti normativi

Il presente documento è redatto ai sensi del Regolamento (UE) 2016/679 (GDPR), del D.Lgs. 196/2003 come modificato dal D.Lgs. 101/2018, della Legge 22 dicembre 2017 n. 219 in materia di consenso informato e del Codice Deontologico dell'Ordine Nazionale dei Biologi.

3. Natura della prestazione sanitaria

Il percorso proposto costituisce una prestazione sanitaria, finalizzata alla valutazione nutrizionale, metabolica e funzionale dell'atleta di endurance.
La prestazione comprende anamnesi clinica, alimentare e sportiva; valutazione della composizione corporea mediante bioimpedenziometria (BIA Biotekna); misure antropometriche e plicometriche; analisi di esami ematochimici forniti dal partecipante; valutazione dei dati di monitoraggio dell'attività fisica e del recupero; restituzione dei risultati con indicazioni nutrizionali e di stile di vita personalizzate.

4. Test del microbiota intestinale

Nell'ambito del percorso può essere effettuato un test del microbiota intestinale mediante campione fecale raccolto autonomamente dal partecipante.
Il campione viene analizzato dal laboratorio Wellmicro®, che opera come titolare autonomo del trattamento dei dati e del campione biologico e fornisce una propria informativa privacy.
La Dott.ssa Florencia Ceppa riceve esclusivamente il referto finale, utilizzato per finalità cliniche.

5. Categorie di dati trattati

- Dati anagrafici e di contatto
- Dati relativi alle abitudini alimentari, allo stile di vita e all'anamnesi clinica
- Dati derivanti da bioimpedenziometria, misure antropometriche e plicometriche
- Referti di laboratorio ed esami diagnostici forniti dal partecipante
- Dati di monitoraggio dell'attività sportiva e del recupero, se condivisi volontariamente

6. Finalità del trattamento dei dati

I dati personali e sanitari sono trattati secondo i principi di liceità, correttezza, trasparenza, minimizzazione e sicurezza.
Le finalità necessarie comprendono l'erogazione della prestazione sanitaria, la gestione del rapporto professionale e gli adempimenti amministrativi, contabili e fiscali.
Con consenso libero, specifico e facoltativo, i dati potranno essere utilizzati esclusivamente in forma anonima e aggregata per finalità di analisi osservazionale, divulgazione scientifica, formazione e sensibilizzazione sul territorio.

7. Natura del conferimento dei dati

Il conferimento dei dati per le finalità sanitarie e amministrative è obbligatorio; l'eventuale rifiuto comporta l'impossibilità di erogare la prestazione richiesta.
Il conferimento dei dati per finalità di ricerca e divulgazione è facoltativo e non condiziona in alcun modo l'accesso o la qualità della prestazione sanitaria.

8. Modalità di trattamento e comunicazione dei dati

Il trattamento dei dati è effettuato sia in forma cartacea sia mediante strumenti informatici protetti da adeguate misure di sicurezza.
I dati non saranno oggetto di diffusione.
Essi potranno essere comunicati esclusivamente a soggetti autorizzati o a professionisti esterni (es. consulente contabile) nei limiti strettamente necessari agli adempimenti di legge.

9. Conservazione dei dati

I dati amministrativi sono conservati per 10 anni, come previsto dalla normativa fiscale.
I dati sanitari sono conservati per il tempo necessario alla tutela professionale.
I dati utilizzati per finalità di ricerca e divulgazione sono conservati esclusivamente in forma anonima.

10. Revoca del consenso e recesso

Il consenso alle finalità facoltative è in ogni momento revocabile, senza pregiudicare la liceità del trattamento effettuato prima della revoca.
In caso di recesso dal percorso, i dati già raccolti potranno essere mantenuti in forma anonima e aggregata per garantire la coerenza delle analisi già effettuate.

11. Diritti dell'interessato

Ai sensi degli artt. 15–22 del Regolamento (UE) 2016/679, l'interessato può esercitare i diritti di accesso, rettifica, cancellazione, limitazione del trattamento, opposizione e revoca del consenso, nonché proporre reclamo al Garante per la protezione dei dati personali.

12. Dichiarazioni di consenso

☐ ACCONSENTO alla prestazione sanitaria descritta nel presente documento.

☐ ACCONSENTO all'utilizzo dei miei dati personali e sanitari, esclusivamente in forma anonima e aggregata, per finalità di analisi osservazionale, divulgazione scientifica, formazione e sensibilizzazione.
☐ NON ACCONSENTO all'utilizzo dei miei dati per finalità di ricerca e divulgazione.

Luogo e data _____

Firma del/della partecipante _____

Il presente documento, debitamente compilato e firmato, dovrà essere consegnato dal/dalla partecipante in occasione della prima visita o inviato tramite posta elettronica all'indirizzo florencia@byotics.it

APPENDICE A
Documentazione richiesta ai fini della partecipazione al progetto di ricerca

Al fine di consentire la corretta valutazione dell'idoneità alla partecipazione al progetto di ricerca **"Studio del microbiota intestinale in atleti di sport di endurance"**, il/la partecipante si impegna a trasmettere alla dott.ssa Florencia Ceppa, **entro la data della prima visita**, la seguente documentazione.

1. Dati anagrafici

- Nome e cognome;
- Indirizzo di residenza;
- Codice fiscale;
- Data di nascita;
- Luogo di nascita.

2. Informazioni sul regime alimentare

- Descrizione del regime alimentare abituale
- Eventuale **scheda nutrizionale/dieta** redatta da un professionista (biologo nutrizionista, dietista, dietologo), se disponibile

Il/la partecipante è inoltre tenuto/a a segnalare eventuali:

- restrizioni alimentari;
- motivazioni etiche, religiose o culturali;
- periodi di digiuno programmati.

3. Esami ematochimici

Si richiede la trasmissione della copia degli **esami ematochimici** più recenti. La documentazione ematochimica sarà utilizzata esclusivamente ai fini della valutazione clinico-nutrizionale, dell'inquadramento metabolico e dell'eventuale identificazione di condizioni che possano influenzare lo stato nutrizionale, infiammatorio o metabolico dell'atleta. In assenza di esami recenti, o qualora i referti disponibili risultino incompleti, non aggiornati o non coerenti con il quadro clinico, la Dott.ssa Florencia Ceppa, a seguito della prima valutazione ambulatoriale, potrà indicare eventuali approfondimenti ematochimici mirati, selezionati sulla base dell'anamnesi, dei segni clinici rilevati e degli obiettivi del percorso nutrizionale personalizzato.

4. <u>Certificazione di idoneità sportiva</u>

- Certificato di idoneità agonistica in corso di validità;
- Test sotto sforzo, preferibilmente comprensivo di:
    - elettrocardiogramma (ECG);
    - eventuale spirometria, se disponibile.

5. <u>Dati di allenamento</u>

- Profilo attivo su piattaforme di monitoraggio dell'attività sportiva, quali:
    - Strava;
    - Garmin;
    - Suunto;
    - o piattaforme equivalenti.

Il/la partecipante autorizza l'utilizzo di tali dati esclusivamente a fini di ricerca, in forma anonima e aggregata.

6. Modalità di invio

La documentazione dovrà essere trasmessa all'ente promotore **<u>entro la data della prima visita</u>**, secondo le modalità comunicate, **<u>tramite posta elettronica all'indirizzo *florencia@byotics.it*</u>**, nel rispetto della normativa vigente in materia di protezione dei dati personali.

Appendix B – Structure of the Baseline Questionnaire

- **Demographic and General Information**

  This section collects basic personal and anthropometric data, including identity, contact details, and reasons for consultation, providing a general overview of the participant's profile.
- **Early Life and Development**

  This section explores early-life factors such as birth conditions, infant feeding, environmental exposure, and early antibiotic use, which may influence long-term microbiota development.
- **Medical History and Gastrointestinal Health**

  This section investigates both gastrointestinal and extra-intestinal health status, including oral, gastric, and intestinal symptoms, bowel habits, and the presence of chronic or diagnosed conditions.
- **Gynecological Health (if applicable)**

  This section includes reproductive and hormonal health information, such as menstrual cycle characteristics, pregnancies, and gynecological conditions.
- **Clinical History and Treatments**

  This section documents past and current diseases, surgical history, allergies, pharmacological treatments, and supplement use, providing a comprehensive clinical background.
- **Dietary Assessment**

  This section evaluates habitual dietary intake through a short-term dietary record and explores dietary patterns, food frequency, eating behaviors, and hydration habits.
- **Lifestyle Factors**

  This section assesses key lifestyle variables, including smoking, alcohol consumption, stress levels, and sleep quality and duration.
- **Physical Activity and Training**

  This section describes the type, frequency, and intensity of physical activity, with particular focus on sport-specific training load.

- **Socio-Behavioral Factors**

  This section examines work or academic activity, as well as social and relational aspects that may influence lifestyle and health behaviors.
- **Environmental Exposure**

  This section considers environmental factors such as contact with animals and characteristics of the domestic environment.
- **Additional Information**

  This section includes supplementary clinical and administrative data, such as recent blood tests, medical certification, notes, and microbiota test identification.

Appendix C – Structure of the Athlete Training and Lifestyle Diary

- **General Information**

  This section records the date and general context of each entry, providing temporal reference for training, dietary, and lifestyle data.

- **Training Sessions**

  This section documents daily training activities, including type of session (e.g., easy run, intervals, strength training), duration, distance, intensity, and any specific notes related to performance or perceived effort.

- **Nutritional Intake**

  This section collects information on daily food intake, including main meals, snacks, and timing of consumption, with particular attention to pre- and post-training nutrition.

- **Hydration and Supplementation**

  This section records fluid intake, type of beverages consumed, and use of dietary supplements or ergogenic aids.

- **Gastrointestinal Symptoms**

  This section monitors the presence of gastrointestinal symptoms, such as bloating, abdominal discomfort, bowel urgency, or altered stool consistency, particularly in relation to training sessions.

- **Recovery and Perceived Fatigue**

  This section evaluates recovery status, perceived fatigue, muscle soreness, and overall readiness to train.

- **Sleep**

  This section includes information on sleep duration and quality, providing insight into recovery and physiological stress.

- **Health Status**

  This section records any illness, injury, or relevant clinical symptoms experienced during the monitoring period.

- **Additional Notes**

  This section allows the athlete to report any relevant observations, including environmental conditions, psychological factors, or deviations from the planned routine.