Evaluación de la interacción entre el microbioma intestinal y la actividad física

Jeimmy Viviana Aya Aldana

Documento de tesis presentado como requisito para optar al título de Doctora en Ciencias Biomédicas y Biológicas

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Evaluación de la interacción entre el microbioma intestinal y la actividad física

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TABLA DE CONTENIDO

1. LISTA DE PUBLICACIONES

Todos los artículos se encuentran anexos a este documento, la información suplementaria y/o tablas serán anexadas en archivos comprimidos siguiendo el número de artículos que se mencionan a continuación:

1. **Aya V,** Flórez A, Pérez L, Ramírez JD. Association between physical activity and changes in intestinal microbiota composition: A systematic review. PLOS ONE. 2021 Feb 25;16(2): e0247039.

2. **Aya V,** Jiménez P, Muñoz E, Ramírez JD. Effects of exercise and physical activity on gut microbiota composition and function in older adults: a systematic review. BMC Geriatr. 2023 Jun 12;23(1):364.

3. **Aya V,** Vega LC, Muñoz E, Muñoz M, López DF, Guzmán MP, et al. Divergent Gut Microbiota: Archaeal and Bacterial Signatures Unveil Unique Patterns in Colombian Cyclists Compared to Weightlifters and Non-Athletes. Adv Biol. 8(6):e2400069.

4. **Aya V,** Pardo D, Vega LC, Cala MP, Ramírez JD**.** Integrating Metagenomics and Metabolomics to Study the Gut Microbiome and Host Relationship in Sports Across Different Energy Systems. (Sometido a Sports Medicine)

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Anexos tesis doctoral Viviana Aya

3. LISTA DE FIGURAS

4. LISTA DE ABREVIATURAS

SCFAS: Ácidos grasos de cadena corta

AGCR: Ácidos grasos de cadena ramificada

AhR: Aryl hydrocarbon Receptor

IgA: Inmunoglobulina A

GPCR: Receptores acoplados a proteína G

LPS: Lipopolisacáridos

GLP-1: Péptido similar al glucagón tipo 1

PYY: Péptido YY

BCAA: Aminoácidos de cadena ramificada

TMAO: Trimetilamina N-óxido

GABA: Ácido gamma-aminobutírico

PCA: Análisis de componentes principales

GC – MS: Cromatografía de Gases - Espectrometría de Masas

IMC: Índice de masa corporal

BCoAT: butiril-CoA acetato CoA-transferasa

RXN: Reacción enzimática

MECIR: Expectativas Metodológicas de las Revisiones de Intervenciones Cochrane

PRISMA: Elementos Preferidos para Informes de Revisiones Sistemáticas y Meta-análisis

PICO/PECO:

PICO: Población, Intervención, Comparación, Resultado

PECO: Población, Exposición, Comparación, Resultado

PROSPERO: Registro Prospectivo Internacional de Revisiones Sistemáticas

EMTRE: Recurso de Capacitación en Medicina Basada en Evidencia

ROBINS-I: Riesgo de Sesgo en Estudios No Aleatorizados - de Intervenciones

IPAQ: Cuestionario Internacional de Actividad Física

DNA: Ácido Desoxirribonucleico

LC – MS: Cromatografía líquida - Espectrometría de Masas

5. RESUMEN

El microbioma se refiere a una compleja red de microorganismos que habitan un nicho específico e interactúan con su hospedero. En el caso del microbioma humano, se pueden identificar diversas comunidades microbianas dependiendo de la ubicación anatómica y de la metodología de análisis utilizada. Entre ellas, el microbioma intestinal ha sido el más estudiado en los últimos años, debido a su estrecha relación con numerosos procesos de salud y enfermedad. Este micro ecosistema está conformado por representantes de los dominios Archaea, Bacteria y Eucaria, así como por una gran diversidad de virus. No solo su composición, sino también su función, particularmente su capacidad para producir metabolitos ha convertido al estudio del microbioma en un campo de investigación multidisciplinario. Desde la fisiología, por ejemplo, se ha intentado comprender cómo los sistemas del cuerpo humano interactúan con estos microorganismos y cómo factores externos, como la dieta, la genética, la ubicación geográfica y el estilo de vida, influyen en este ecosistema. Entre estos factores, la actividad física ocupa un lugar destacado.

En los últimos años, ha surgido un campo de investigación que se centra en estudiar la relación y los posibles efectos de la actividad física sobre el microbioma intestinal. Inicialmente, se planteó la hipótesis de que la actividad física podría generar un aumento en la diversidad bacteriana y provocar cambios significativos en la estructura de los microorganismos del sistema digestivo, de manera similar a los efectos ya ampliamente descritos de la dieta. Sin embargo, los resultados obtenidos en la literatura no han sido concluyentes ni fáciles de comparar, debido a la heterogeneidad de los estudios. Estos incluyen diseños tanto longitudinales como transversales, diferentes tipos e intensidades de actividad física, diversidad de poblaciones, y una amplia variedad de técnicas utilizadas para describir las comunidades microbianas. Además, la mayoría de los estudios se han centrado en sujetos que realizan actividades donde predomina el entrenamiento de resistencia cardiovascular, como el ciclismo o el atletismo, dejando de lado actividades que promuevan el desarrollo de la fuerza y la masa muscular.

El objetivo general de este trabajo fue evaluar la relación entre el microbioma intestinal y la actividad física mediante revisiones sistemáticas de la literatura y un estudio transversal en humanos a través de un enfoque metagenómico y metabolómico. Para lograr este objetivo, el trabajo se dividió en dos partes. La primera consistió en una revisión exhaustiva de la literatura sobre la relación entre el microbioma intestinal y la actividad física. La segunda parte se centró en un estudio transversal enfocado en deportistas colombianos, utilizando aproximaciones metagenómicas y metabolómicas para explorar dicha relación.

Ante la necesidad de comprender si el microbioma intestinal es modificable mediante la actividad física y cómo estos cambios pueden estar influenciados por factores como el tipo, la intensidad, la duración del ejercicio y los métodos de medición, el primer capítulo de esta tesis se centró en evaluar dicha relación mediante revisiones sistemáticas de la literatura. Se llevaron a cabo dos revisiones: una enfocada en adultos y otra en adultos mayores, abarcando un total de 29 estudios (diez estudios transversales, siete longitudinales y tres ensayos controlados aleatorizados).

En la primera revisión, los estudios en adultos aparentemente sanos revelaron que la actividad física se asocia con cambios moderados en la diversidad bacteriana y un aumento en la abundancia de bacterias beneficiosas, como *Akkermansia* y *Bifidobacterium*. Sin embargo, los resultados variaron significativamente según la intensidad y duración del ejercicio, dificultando conclusiones uniformes.

Por otro lado, en la segunda revisión, se evaluó la relación en adultos mayores. Los hallazgos indicaron que programas de ejercicio de al menos cinco semanas podrían inducir cambios significativos en la composición del microbioma, con un incremento en la abundancia de bacterias del género Firmicutes, asociado a mejoras en la función metabólica. A pesar de estos hallazgos, la evidencia en adultos mayores sigue siendo limitada y altamente dependiente del tipo e intensidad del ejercicio realizado.

Como se mencionó anteriormente, el microbioma intestinal posee una notable capacidad para producir una amplia variedad de metabolitos primarios y participar en la síntesis o degradación de compuestos derivados de la dieta. Estos metabolitos suelen actuar como moléculas señalizadoras, facilitando la comunicación entre el microbioma y el huésped. Sin embargo, para explorar eficazmente estas interacciones, es necesario implementar enfoques y herramientas ómicas que permitan analizar la información en distintos niveles, desde la estructura hasta la función de los microorganismos presentes en el sistema digestivo.

En cuanto a la influencia de la actividad física sobre las bacterias, arqueas, virus y diversos eucariotas residentes en el sistema digestivo, investigaciones previas sugieren la posibilidad de establecer una conexión más significativa entre la actividad física y el microbioma a nivel funcional. Por ello, en el segundo y tercer capítulo de este trabajo se exploró la relación entre el microbioma intestinal y los metabolitos asociados con el ambiente intestinal en deportistas colombianos de las disciplinas de halterofilia y ciclismo de ruta, las cuales implican procesos adaptativos orientados a la fuerza y la resistencia cardiovascular. Se emplearon herramientas de metagenómica y metabolómica para caracterizar la población microbiana, perfilar su función genómica y cuantificar los metabolitos en muestras fecales y plasma, relacionados con su actividad deportiva.

En síntesis, el segundo capítulo de esta tesis se centró en la caracterización del perfil taxonómico del microbioma intestinal de deportistas colombianos en las disciplinas de ciclismo de ruta y levantamiento de pesas. Este análisis permitió identificar patrones microbianos divergentes entre los grupos de ciclistas, halterofilia y no deportistas, destacando diferencias específicas en la composición y abundancia de microorganismos pertenecientes a los dominios Bacteria y Archaea. Además, se identificaron virus específicos del tipo Crass-like, los cuales fueron más abundantes en los ciclistas.

Finalmente, el tercer capítulo permitió evaluar no solo la abundancia de genes y rutas metabólicas relacionadas con el microbioma intestinal, sino también identificar metabolitos clave mediante el uso de metabolómica no dirigida. Este enfoque integrativo utilizó herramientas de metagenómica y metabolómica para explorar la relación entre el microbioma y la actividad física en los deportistas de disciplinas con diferentes sistemas

energéticos (halterofilia y ciclismo). Los resultados indicaron la presencia de rutas metabólicas específicas, como la biosíntesis de aminoácidos ramificados (valina, leucina e isoleucina) y la biosíntesis de arginina, que podrían estar asociadas con el rendimiento deportivo. Además, el análisis metabolómico mostró un enriquecimiento de metabolitos relacionados con la actividad física, especialmente aquellos involucrados en el metabolismo de ácidos grasos y aminoácidos.

Los hallazgos presentados a lo largo de los tres capítulos de esta tesis ofrecen una visión integral de la relación entre la actividad física y el microbioma intestinal. En el primer capítulo, se identificaron diferencias clave en la composición del microbioma entre atletas y no atletas, sugiriendo que el nivel de actividad física influye significativamente en la diversidad y estructura microbiana. Estos resultados se complementaron en el segundo capítulo, donde se encontraron firmas microbianas distintivas entre ciclistas y levantadores de pesas, lo que indica que las distintas modalidades deportivas, con sus demandas energéticas específicas, pueden modular el microbioma de manera diferencial. Finalmente, el tercer capítulo mostró que estas variaciones en el microbioma también se reflejan en el perfil metabólico, con posibles implicaciones en el rendimiento físico, especialmente en el metabolismo de ácidos grasos y aminoácidos. En resumen, estos resultados refuerzan la idea de que la actividad física no solo modula la composición del microbioma intestinal, sino también sus funciones metabólicas, lo cual podría influir directamente en el rendimiento deportivo y la salud metabólica. No obstante, la falta de datos sobre hábitos dietéticos y fisiológicos limita la capacidad de contextualizar plenamente estos cambios.

Futuros estudios deberían abordar las limitaciones actuales, como la falta de datos dietéticos y fisiológicos, e incorporar enfoques multiómicos que permitan explorar con mayor precisión las complejas interacciones entre el microbioma, la actividad física y el metabolismo del hospedador. Además, sería recomendable que las investigaciones futuras se enfoquen en ensayos clínicos controlados, con el fin de esclarecer la relación causal entre la actividad física y los cambios en el microbioma intestinal. La integración de datos multiómicos—que incluyan el microbioma, genómica, metabolómica, y otros—podría aportar una visión más completa de los efectos del ejercicio sobre la salud y el rendimiento. Además, explorar otros dominios microbianos (virus, hongos, arqueas) y su interacción con la microbiota bacteriana en respuesta al ejercicio físico representa una prometedora área de investigación. Considerar factores como la dieta, el sueño y el estilo de vida en general también será esencial para entender mejor la influencia del ejercicio en el microbioma y cómo optimizar intervenciones de salud basadas en la actividad física.

6. MARCO TEÓRICO

Microbioma y microbioma intestinal

La diversidad de microorganismos que reside en el tracto gastrointestinal humano constituye la microbiota intestinal (Figura 1). Esta comunidad alberga representantes microscópicos de cada uno de los dominios de la vida: procariotas, eucariotas y arqueas, junto con una cantidad significativa de virus [1,2]. Dado que la mayoría de estos comensales son bacterias, el enfoque en el estudio de la microbiota intestinal humana se ha centrado tradicionalmente en el estudio de Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria y Verrucomicrobia, entre otros phyla [3]. Sin embargo, gracias al desarrollo de técnicas de secuenciación genómica y al fortalecimiento de bases de datos de eucariotas y virus, en la actualidad se cuenta con una visión más completa de esta comunidad [4]. Así mismo, la diversidad genética de los microorganismos comensales puede superar hasta 150 veces la humana, lo que se traduce en una amplia gama de actividades metabólicas [5]; esta relación se entiende como mutualismo dado que tanto hospedero como microbioma reciben beneficios [6]. Finalmente, el microbioma abarca la diversidad genética y funcional, así como el intercambio de información con el hospedero [7] lo que lo convierte en un concepto más holístico que el de microbiota [8,9].

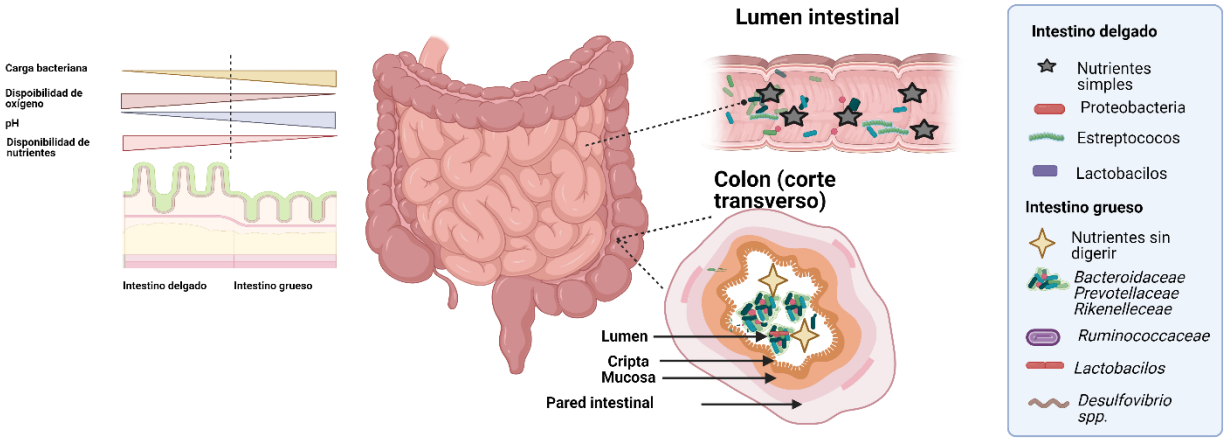

Figura 1: Distribución espacial de las diferentes bacterias que pueden colonizar segmentos del tracto digestivo. Los cambios en la composición y abundancia de microorganismos están mediados por las condiciones del ambiente intestinal (cantidad de oxígeno, cambios en el pH, disponibilidad de moco) y por la disponibilidad de nutrientes. La interacción entre microorganismos y células del epitelio beneficia al hospedero a través de distintos mecanismos. Imagen propia basada en las referencias [8, 39].

Funciones del microbioma intestinal

El microbioma intestinal desempeña una serie de funciones que son de vital importancia para el hospedero [11] a continuación se listan las funciones más relevantes de este nicho ecológico (Figura 2):

a) Función metabólica: La composición del microbioma intestinal se ve afectada principalmente por la dieta, lo que conlleva cambios significativos en este ecosistema. La fermentación de los productos alimenticios en el tracto gastrointestinal produce ácidos grasos de cadena corta (SCFAs) [12], como el propionato, acetato y butirato, que son utilizados por las bacterias como fuente de alimento [13]. Estos SCFAs desempeñan diversas funciones metabólicas, como inducir la gluconeogénesis, regular la glucosa, estimular la secreción de hormonas y afectar la termogénesis y oxidación lipídica [14]. La fermentación proteolítica en el colon produce ácidos grasos de cadena ramificada (AGCR), indoles, sulfato de hidrógeno y amonio, cuya variabilidad se ha relacionado con la salud y la inflamación. La transformación del triptófano en ligandos AhR, indoles y serotonina por el microbioma intestinal tiene importantes implicaciones en el sistema inmune, nervioso y digestivo. Además, el microbioma participa en el metabolismo del colesterol y en la síntesis de vitaminas esenciales, principalmente del complejo B [15–17].

b) Función de protección y mantenimiento: Parte de la relación mutualista entre microbioma y humano se sustenta en la función protectora que ejercen las bacterias en las paredes del intestino [18], debido a la comunicación directa con el revestimiento epitelial. Algunas bacterias que componen el microbioma secretan proteínas que estimulan la producción de mucinas por parte de las células humanas, aumentando así la disponibilidad de moco que funge como barrera contra patógenos y compuestos derivados de microorganismos, como por ejemplo los lipopolisacáridos (Figura 2). Las mucinas son familias de proteínas secretadas por las células globet del intestino y recubren de manera exitosa las superficies epiteliales; la disponibilidad y composición de este gel protector varía considerablemente entre intestino delgado y grueso [19]. Esta barrera cumple con varias funciones, por una parte evita la traslocación de potenciales patógenos al torrente sanguíneo [19] y por otro lado debido a su composición rica en glucanos provee una fuente estable de energía para la bacterias comensales del intestino [20,21].

c) Función de defensa: Gran parte de la respuesta inmune en mamíferos es mediada por el ambiente intestinal [21], la lámina propia del intestino es un tejido altamente vascularizado y rico en linfocitos; una amplia diversidad de células especializadas se encuentran agrupadas en este epitelio, algunas de ellas son las células dendríticas, células tipo B, y células $CD4^+$ T [22]. Ya que estás guardan una relación directa con la composición y productos de la microbiota, los procesos de colonización y establecimiento desde la primera infancia son de suma importancia para este ecosistema [23]. La interacción entre células del sistema inmune, intestinales y microbioma (Figura 2) permite el establecimiento de la respuesta inmune; esto es posible gracias a diferentes mecanismos. Uno de ellos es la secreción de inmunoglobulinas (IgA) que son liberadas a la luz intestinal por parte de linfocitos [24], la producción de SCFAS contribuye a la producción de receptores transmembrana de diferente tipo (denominadas GPCR por sus siglas en inglés) lo que

aumenta la sensibilidad de células dendríticas para detectar patógenos y posibles amenazas al ser neutralizadas [22]. De igual manera el mantenimiento de un microbioma equilibrado permite la neutralización de posibles patógenos mediante mecanismos de control inter dominio [25]. Además de los SCFAS, el intercambio de información entre microbioma y sistema inmune también puede ocurrir gracias a neurotransmisores, ácidos biliares, productos de la degradación de colina, ácidos aromáticos y fenólicos, entre otros [26–28].

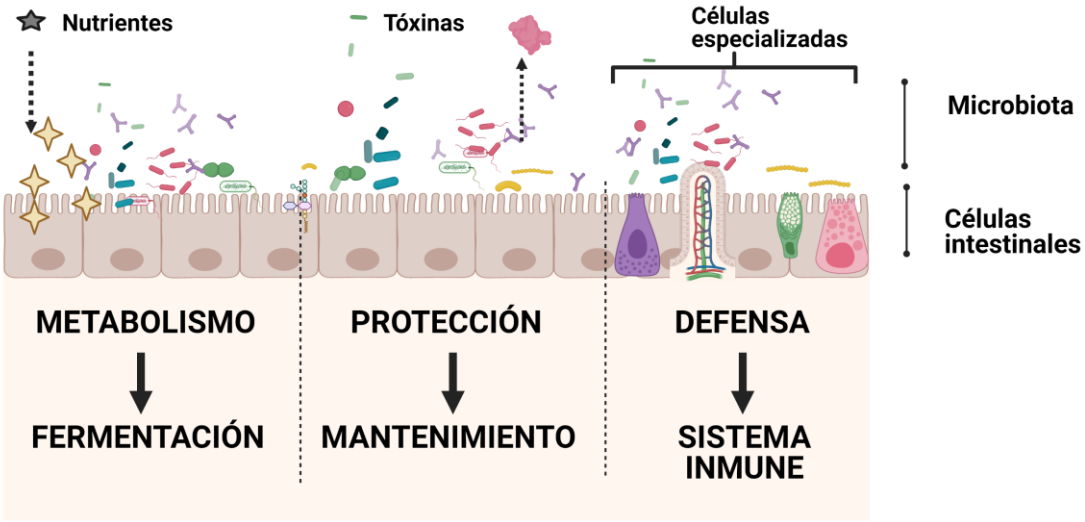

Figura 2: Funciones principales del microbioma intestinal humano. El conjunto de microorganismos que habita de manera simbiótica en el intestino cumple con diversas funciones que benefician al hospedero. Imagen propia basada en las referencias [26–28].

Concretamente el microbioma intestinal puede actuar de manera local y sistémica, como se puede observar en la figura 3, por consiguiente, en la actualidad se han identificado diferentes rutas metabólicas que podrían explicar la comunicación entre microbioma y sistemas diferentes al gastrointestinal [29], lo que explica en gran parte la diversidad funcional que alberga este nicho ecológico.

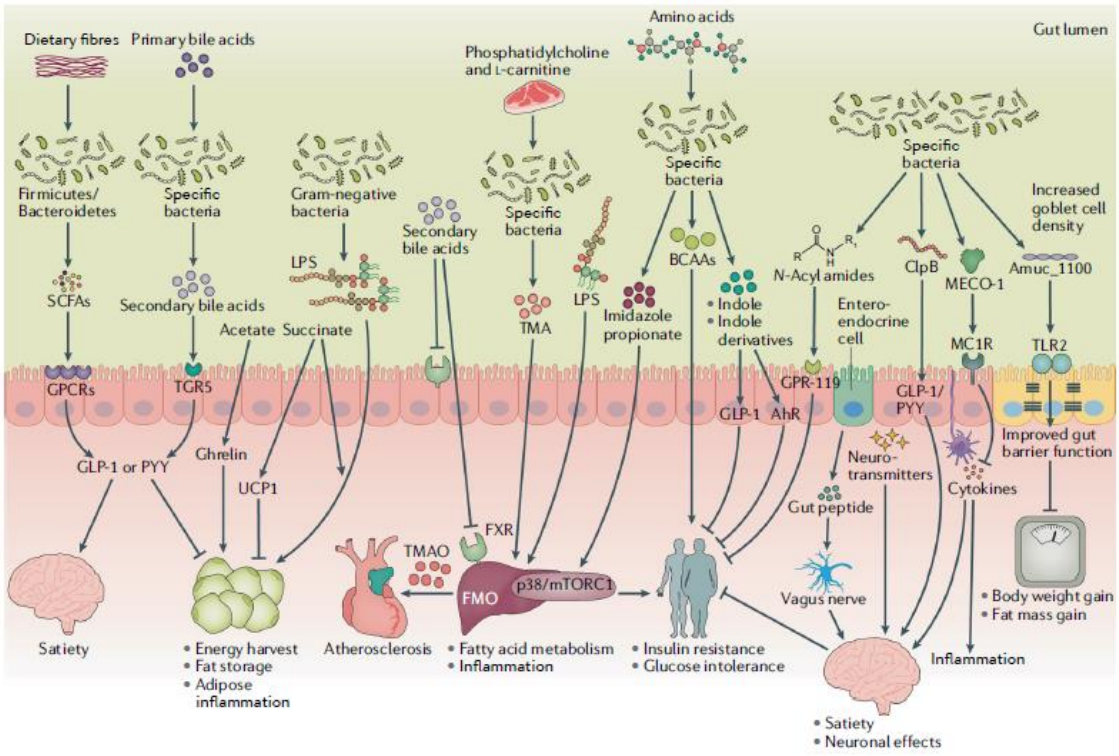

Figura 3: Acción local y multisistémica del microbioma intestinal. Tomado de Fan, Y., & Pedersen, O. (2020) Referencia [29]. Los compuestos microbianos intestinales influyen en la homeostasis energética del huésped, la inflamación, la regulación de la glucosa, la secreción de hormonas y la sensibilidad a la insulina. La fermentación de la fibra dietética por bacterias intestinales, como Firmicutes y Bacteroidetes, produce SCFAS que afectan el metabolismo humano activando receptores acoplados a proteínas G en células entero-endocrinas. Estos metabolitos microbianos estimulan la liberación de péptidos como GLP-1 y PYY, con efectos en la biosíntesis de insulina y la saciedad. Además, otros compuestos microbianos, como el succinato y LPS, tienen efectos proinflamatorios que contribuyen a la inflamación del tejido adiposo y la resistencia a la insulina. También se destacan los efectos de metabolitos derivados de BCAA, TMAO y propionato de imidazol en la resistencia a la insulina y la aterosclerosis. Otros productos microbianos modulan el apetito y la función de la barrera intestinal, como lo hace la proteína Amuc_1100 de *Akkermansia muciniphila*. La microbiota intestinal también sintetiza neurotransmisores que influyen en el metabolismo del huésped, como la serotonina y el GABA [29].

Factores que modifican el microbioma intestinal

Gracias a que durante los últimos años se han conducido estudios que han permitido establecer la relación entre cambios en el microbioma y procesos de salud – enfermedad [30], estilos de vida y cambios durante las diferentes etapas del ciclo vital [23]; una serie de factores moduladores del microbioma intestinal han sido descritos. Investigaciones al respecto indican que la microbiota intestinal en individuos sanos ha mostrado ser estable, especialmente cuando hay ausencia de manipulación clínica (por ejemplo, uso indiscriminado de antibióticos) y en general hábitos de vida saludable, como una dieta adecuada y actividad física de moderada a vigorosa intensidad [31].

Un balance saludable de bacterias en el tracto digestivo asegura que el microbioma trabaje en un ambiente simbiótico con el hospedero, no obstante, cambios en la diversidad podrían conducir a una reducción en la abundancia de bacterias benéficas y aumento en la prevalencia de microorganismos potencialmente patógenos, a lo que se le ha denominado "disbiosis". Por su parte la disbiosis ha sido vinculada con diversas enfermedades que van desde la primera infancia hasta la vejez [32].

En vista de su relación con diversas patologías [33] y gracias a la alta variabilidad entre sujetos, la diversidad de microorganismos que conforman la microbiota puede cambiar significativamente de una persona a otra [34]; razón por la cual consorcios y grupos de investigación han coordinado esfuerzos para generar bases de datos e información relevante sobre este micro ecosistema [35,36]. En la figura 4 se presenta una serie de factores modulantes que son inherentes a la biología del humano, tales como la genética, estados patológicos y sexo; así mismo se presentan otros factores de tipo extrínsecos que pueden impactar de manera positiva o negativa el microbioma: dieta, sueño, nivel de estrés, ubicación geográfica y nivel de actividad física.

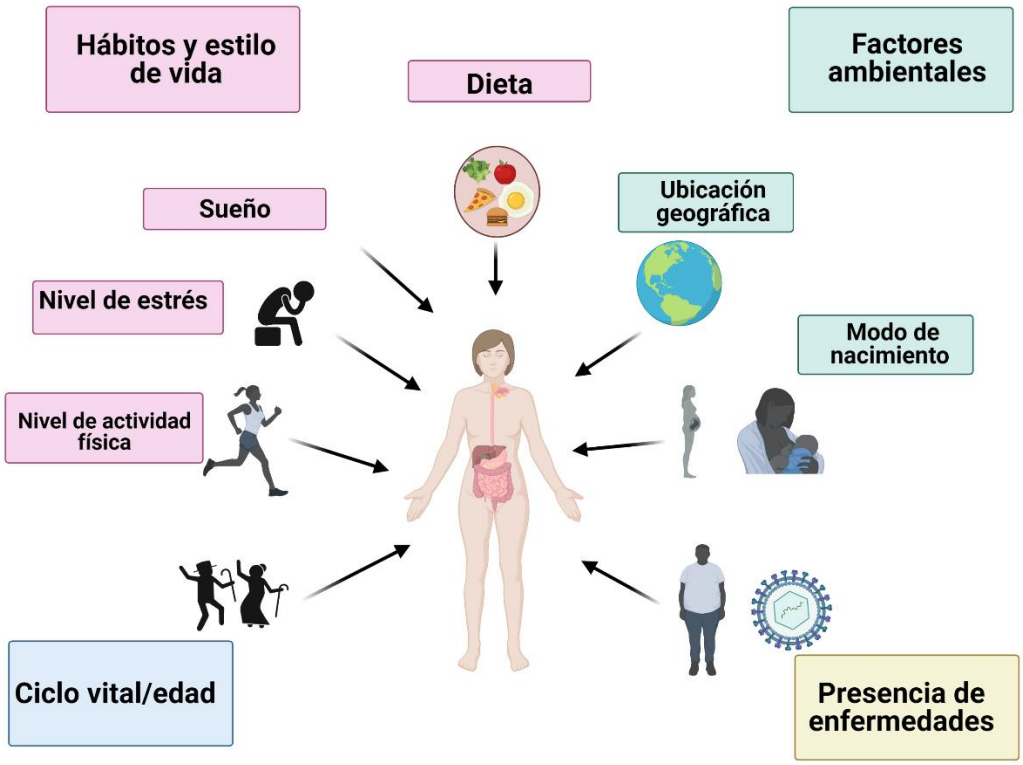

Figura 4: Factores internos y externos que modulan el microbioma intestinal. Imagen de creación propia basada en las referencias [2,80,81]. **a) Hábitos y estilos de vida**: Los factores como la dieta, la actividad física, el consumo de alcohol, tabaco y el uso de medicamentos (incluyendo antibióticos) influyen directamente en la composición de la microbiota intestinal. Un estilo de vida saludable favorece la diversidad microbiana, mientras que hábitos poco saludables pueden

reducirla. **b) Ciclo de vida**: El microbioma intestinal cambia a lo largo de las etapas de vida del ser humano, desde el nacimiento hasta la vejez. **c) Factores ambientales**: El entorno en el que vivimos, incluidos aspectos como la exposición a contaminantes, el clima, la altitud y el acceso a espacios naturales, también puede modular la composición del microbioma. Factores como el tipo de parto, la lactancia y los cambios hormonales o metabólicos durante la vida influyen en su evolución y función. Estos factores externos interactúan con el cuerpo para influir en la diversidad y función de las bacterias intestinales. **d) Presencia de enfermedades**: Las enfermedades crónicas, inflamatorias y metabólicas, como la obesidad, diabetes y enfermedades intestinales, alteran el equilibrio del microbioma. Las enfermedades infecciosas, causadas por microorganismos patógenos como bacterias, virus, hongos y parásitos, pueden alterar significativamente la composición del microbioma intestinal. Infecciones gastrointestinales y enfermedades virales también afectan este equilibrio, modificando la relación entre el huésped y su microbiota, lo que puede tener consecuencias para la salud.

Estudio de la relación entre actividad física y microbioma intestinal

Los beneficios de realizar actividad física han sido ampliamente estudiados en los últimos años [37], aumentar el nivel de actividad física y realizar ejercicio de manera regular traen consigo consecuencias positivas en las alteraciones metabólicas mencionadas anteriormente [38]; lo que representa un tratamiento costo efectivo para revertir muchas de las complicaciones que aquejan a la población mundial [39]. El microbioma intestinal impacta al hospedero de manera dependiente al estado de salud, razón por la cual aumentar el nivel de actividad física podría conferir beneficios a este nicho ecológico, la comunidad de microorganismos que componen el ambiente intestinal puede modificarse o no a través del ejercicio en dependencia de diversos factores [40,41]. A continuación, se presenta una serie de hallazgos que respaldan la evidencia de la relación entre el microbioma intestinal y la actividad física en modelos murinos (Figura 5).

Algunas intervenciones basadas en reducción de calorías y/o manipulación de la dieta en modelos animales, niegan que el efecto del ejercicio físico se centre únicamente en el aumento de Alfa y Beta diversidad, [42–44] por lo que esto representa un factor de confusión importante que debe ser tenido en cuenta. Por otro lado, el ejercicio parece aumentar la abundancia de ciertas bacterias, principalmente a nivel de género y phyla, como *Odoribacter*, *Akkermansia*, *Faecalibacterium prausnitzi*, *Allobaculum spp*. y *Clostridium spp* [40,41,44]. Algunos de estos microorganismos han sido reportados como simbióticos o benéficos, ya que su genoma codifica para la producción de ácidos grasos de cadena corta (SCFAS), principalmente butirato y acetato. Estos metabolitos pueden conferir protección a las paredes intestinales y aumentar la permeabilidad de la barrera intestinal, lo que a su vez incrementa la recolección de energía proveniente de la dieta, entre otras funciones que pueden beneficiar al hospedador [45]. Sin embargo, aún no está claro si el ejercicio por sí solo puede mejorar y/o modificar la capacidad metabólica de las bacterias mencionadas [46].

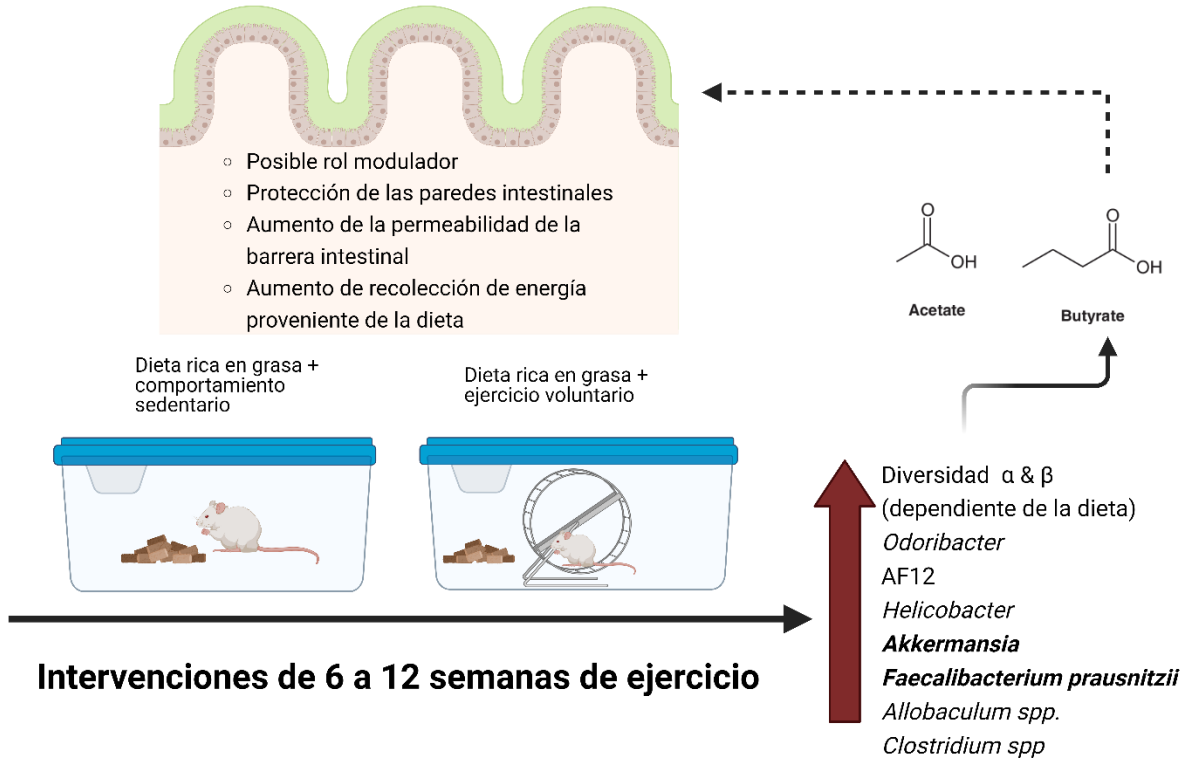

Figura 5: Síntesis de la evidencia, relación ejercicio físico y microbioma intestinal estudiada en modelos murinos. Imagen de creación propia basada en las referencias [40,42,43,43,47–49,49,50]. Diversos estudias evidencian que el ejercicio físico modula el microbioma intestinal en modelos animales con diferentes características. Se observó un aumento en la abundancia de bacterias beneficiosas, como *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, asociadas con la mejora en la salud intestinal. Además, se registraron cambios significativos en las diversidades alfa y beta del microbioma, las cuales variaron según la dieta de los animales. Estos resultados sugieren que el ejercicio físico puede tener un papel modulador importante en la composición y diversidad del microbioma intestinal, dependiendo del contexto dietético.

En relación con otros factores asociados al hospedero, como la edad, se evidencian resultados diferenciales; ratones jóvenes y con peso adecuado tuvieron una microbiota más enriquecida y con mayor abundancia de especies que su contraparte más vieja [51]. En un experimento diferente llevado a cabo en ratones envejecidos se identificó un aumento significativo en la abundancia relativa de *Bacteroides* ($p = 0.03$), así como una reducción en la abundancia relativa de las bacterias *Helicobacter* y *Lactobacillus* ($p = \leq 0.05$) tras un régimen de entrenamiento de cuatro semanas [50]

De igual manera, otros modelos murinos sugieren que el ejercicio puede revertir estados patológicos a través de la microbiota intestinal y que dichas modificaciones podrían ser identificadas en ratones receptores de trasplante de materia fecal. Un estudio llevado a cabo por Allen et al., reporta que el ejercicio genera modificaciones en la microbiota que pueden ser trasferidas a ratones gnotobióticos y que incluso estas podrían atenuar la respuesta a la inducción de estados inflamatorios como la colitis [52]. Sin embargo, se presentan contradicciones con otros

experimentos donde no se reportan diferencias significativas en la comunidad de microorganismos de ratones con otro tipo de condición como diabetes [48], o menopausia [53].
Por otro lado, los estudios sugieren que variables propias del ejercicio físico como intensidad, duración y tipo de actividad tienen un efecto diferencial en las bacterias que componen la microbiota [43,54,55]. En animales, implementar un régimen de entrenamiento mayor a cuatro semanas ha mostrado tener un efecto protector tras la inducción de ciertas condiciones patológicas o exposición a ambientes tóxicos, con cambios considerables en la microbiota intestinal, principalmente relacionados al aumento en la abundancia de taxones reportados como benéficos [56]. En estudios donde los animales son analizados según la forma de realizar las actividades, es decir de manera voluntaria o forzados por algún tipo de estímulo externo, se evidencian diferencias significativas en la riqueza de especies, siendo menor en condiciones estresantes y forzadas [49].

Aunque el ejercicio puede potenciar algunas funciones del ambiente intestinal y modificar el microbioma, hay una serie de alteraciones negativas que están relacionadas con altos niveles de entrenamiento; sobrecarga y fatiga que pueden repercutir en el estado de salud, así mismo, algunas dolencias estomacales, infecciones intestinales y problemas relacionados al tracto gastro intestinal son frecuentes en deportistas de alto rendimiento [57,58]. Estudios en modelo animales han demostrado que superar el umbral físico, es decir sobrepasar los límites fisiológicos del cuerpo durante actividades de alta demanda energética pueden desencadenar en perdida de la integridad de la barrera intestinal, inflamación, aumento de lipopolisacáridos (LPS) en sangre, entre otros [59]. Fisiológicamente el ejercicio de alta intensidad aumenta el flujo sanguíneo en el musculo esquelético, mientras que lo disminuye en el hígado y en la vena portal, de hecho un aumento sostenido de este estado de hipoxia puede llegar a inducir daño hepático irreversible [60]. Esto es debido a que durante el estado de fatiga gran cantidad de ácido láctico, amonio y nitrógeno úrico se acumula en suero sanguíneo y músculos. La disminución y agotamiento de reservas de glucógeno son evidentes durante la contracción sostenida del músculo, lo que representa un problema importante para la población deportista debido a la posibilidad de no rendir lo suficiente en una competencia o inducir daños importantes a diferentes niveles [61]; el ejercicio excesivo ha sido asociado además con inmunodepresión, aumentando la posibilidad de adquirir infecciones [62].

Actualmente no es posible establecer si estos cambios son en parte mediados por el microbioma intestinal o si por el contrario el aumento de la intensidad conlleva cambios significativos. Comparaciones entre ratas con alto nivel cardiorrespiratorio señalan una composición de la microbiota diferente, con una marcada disminución del phyla Actinobacteria ($p = 0.041$), de la familia Veillonellaceae ($p = 0.007$) y de Coriobacteriaceae ($p = 0.041$); así como del género *Phascolarctobacterium* ($p = 0.011$) y de *Ruminococcus* ($p = 0.019$), de igual manera se evidencia un aumento de *Lactobacillus* ($p = 0.043$) [114]. Análisis de diversidad Alfa en muestras fecales revelan que el índice Simpson disminuye considerablemente en ratones sometidos a protocolos de sobretratamiento ($p = \leq 0.05$), estos resultados fueron confirmados mediante análisis estadístico como PCA donde se registra una diferencia importante entre el grupo control (sedentario) y los animales ejercitados ($p = \leq 0.01$), además se reporta un conjunto de alteraciones en células del sistema inmune [63].

Ejercicio físico y metabolismo a la luz del microbioma intestinal en humanos

En vista que el ejercicio físico puede mejorar la motilidad intestinal, lo que en consecuencia reduce el tiempo de contacto de materia fecal con paredes del intestino [64] y que intensidades moderadas y descanso oportuno permiten un aumento en la expresión de proteínas de unión en las células que conforman el epitelio intestinal, disminuyendo la probabilidad de traslocación de patógenos o derivados como los LPS al torrente sanguíneo [65]. La actividad física constante ha sido relacionada con beneficios sistémicos que pueden estar mediados por la actividad metabólica del microbioma, específicamente el ejercicio físico reduce significativamente el flujo sanguíneo en el colón lo que disminuye la disponibilidad de oxígeno en esta parte de tracto digestivo [66]; una condición necesaria para el aumento y expansión de microorganismos en este ambiente (Figura 1). Por esta razón algunas investigaciones han buscado determinar si el esfuerzo físico confiere cambios en diversidad y abundancia de bacterias y si dichos cambios podrían ser rastreados más allá del eje microbioma – intestino. Algunos autores señalan modificaciones en el microbioma intestinal como un beneficio más de realizar ejercicio físico ya que se inducen cambios en bacterias que no requieran el oxígeno como aceptor final en la cadena de electrones [41]. Sin embargo, los cambios evidenciados en estudios que involucran tanto animales como humanos no son del todo concluyentes [54], por lo tanto, el impacto del ejercicio en la composición y estructura del microbioma intestinal aún no está completamente definido.

Es importante resaltar la diferencia en las muestras incluidas en estudios dirigidos a humanos: adultos mayores [67,68], adultos jóvenes [69,70] y adolescentes [71]; algunos estudios se han centrado en comparar grupos poblacionales de personas activas con sedentarios [72–74], mientras que otros autores han optado por un diseño de tipo longitudinal con el objetivo de examinar cambios en el microbioma tras un periodo de entrenamiento; dichos experimentos han incluido el ejercicio de tipo aeróbico [75] con tiempos de intervención e intensidades diferentes [76]. De igual manera se han incluido personas aparentemente sanas [69,70] o con algún tipo de condición como cáncer [77,78], obesidad [75,76] y diabetes [79].

Algunos de los resultados en este campo, involucran cambios en la abundancia de taxones bacterianos como *Lactobacillus*, *Bifidobacterium*, y *Akkermansia* que tienden a aumentar con la práctica de ejercicio físico tanto en ratones como en humanos; mientras que otros grupos taxonómicos como Proteobacteria, *Turicibacter*, Rikenellaceae tienden a disminuir [80]. Como es de esperarse muy pocos estudios han ampliado los resultados a otro tipo de microorganismos como virus [81] y arqueas [82], mientras que el papel de helmintos y otros eucariotas no ha sido identificado. De igual manera, variables relacionadas al tiempo de intervención podrían modular la respuesta al ejercicio físico ya que las bacterias comensales del tracto digestivo no sufren cambios drásticos tras seis semanas de ejercicio predominantemente aeróbico, pero si modifican la concentración de ácidos grasos de cadena corta y la expresión génica que los regula [76]. El ejercicio puede conferir ventajas metabólicas al hospedero a través del microbioma intestinal, a pesar que indicadores de diversidad Alfa o Beta no sean modificables en todos los casos, algunos resultados sugieren que el establecimiento de una comunidad de microorganismos más eficiente para la producción de energía por medio de la glicolisis y fosforilación oxidativa son resultado del ejercicio [66,83], lo que podría contribuir a beneficios como mayor rango en la degradación de carbohidratos, y reducción de masa grasa.

Los autores sugieren que estos resultados explican la prevención de enfermedades, vía el eje músculo – intestino [84,85] y en especial gracias a la producción de ácidos grasos de cadena corta que pueden alcanzar la circulación e impactar otros órganos como el hígado y el músculo [86]. Algunos cambios específicos y a nivel de especie identifican que el hecho de realizar actividad física en humanos puede modular el ambiente intestinal gracias al aumento en la abundancia de bacterias productoras de butirato como *Faecalibacterium prausnitzii* y *Akkermancia muciniphila* [87]. Sin embargo, no es posible identificar un rol activo de los microorganismos en el proceso de adaptación y respuesta al esfuerzo físico en humanos, algunos autores sugieren que este puede estar más relacionado con modificantes taxonómicas y metabólicas que con indicadores de estructura y composición [83,83,85,88,89].

Gracias a que los SCFAs han sido ampliamente estudiados debido a sus beneficios en el sistema digestivo, el butirato [90] y acetato [91] son los bio-compuestos más reportados en este campo de investigación. El ejercicio físico ha sido relacionado con el aumento de bacterias productoras de butirato [92], sin embargo, el mecanismo de acción no ha sido descrito del todo principalmente porque no existe claridad sobre si la actividad física aumenta la expresión de genes en dichas bacterias [49] o si por el contrario es la abundancia de las mismas las que permiten identificar mayor cantidad de este compuesto volátil en el colón [90], desafortunadamente muy pocas investigaciones en humanos han explorado esta potencial relación entre metabolismo, microbioma y actividad física por lo que posibles mecanismos de acción permanecen sin ser descritos.

Estudios previos han demostrado que el ejercicio en individuos físicamente inactivos produce cambios en la composición del microbioma y mejora la síntesis de metabolitos asociados al ambiente intestinal. Allen y col. [88] utilizaron cromatografía de gases (CG-MS) para cuantificar los SCFAs de muestras fecales de personas que comenzaron un programa de 6 semanas de ejercicio principalmente aeróbico, los participantes que se diferenciaron principalmente por el IMC (delgado y obeso) mostraron un aumento significativo en la concentración de SCFAs después del período de entrenamiento. Los aumentos significativos en las bacterias productoras de butirato, especialmente en personas delgadas, como *Roseburia spp*, *Lachnospira spp*, Lachnospriaceae, *Clostridiales* y *Faecalibacterium* se han correlacionaron positivamente con los cambios en las concentraciones de butirato y los genes de butiril-CoA: acetato CoA-transferasa (BCoAT) [45,93].

De igual manera, realizar actividades de alta demanda energética (por ejemplo, correr una media maratón) también se ha asociado con modificaciones en los metabolitos relacionados con los microorganismos intestinales. Un estudio que utilizó análisis metabolómico con cromatografía líquida (LC – MS) de muestras antes y después de un evento de medio maratón en Shanghai informó un aumento en la concentración de aproximadamente 40 metabolitos, principalmente ácidos orgánicos. Las rutas metabólicas con mayores cambios tras la carrera fueron las pentosas fosfato más enriquecidas con un valor de $q = 0,0071$, mientras que la biosíntesis de fenilalanina, tirosina y triptófano se redujo de manera significativa [94]. Estos datos permiten identificar que el estudio del microbioma intestinal en torno a la actividad física necesita de la incorporación de herramientas moleculares ya que la identificación de miembros de interés puede no ser suficiente, el perfilamiento de compuestos metabólicos asociados a la alta actividad que tienen los microorganismos en el intestino y que pueden impactar de diferentes manera el metabolismo del hospedero brinda una posible ruta para comprender esta relación.

Realizar actividades de alta demanda energética, como correr una media maratón, se ha asociado con modificaciones en metabolitos que interactúan con los microorganismos intestinales. Un estudio realizado por Zhao y colaboradores [94], utilizó cromatografía líquida acoplada a espectrometría de masas (LC-MS), donde se reportó un aumento en la concentración de aproximadamente 40 metabolitos después de una media maratón, principalmente ácidos orgánicos. Las rutas metabólicas más afectadas incluyeron las pentosas fosfato, mientras que la biosíntesis de aminoácidos esenciales como fenilalanina, tirosina y triptófano se redujo significativamente [94].

Además, el papel del microbioma intestinal en la modulación del metabolismo del hospedador y el rendimiento deportivo ha sido un área de creciente interés. Estudios recientes refuerzan la importancia de integrar enfoques ómicos para obtener una visión más completa de las interacciones entre el microbioma y el metabolismo [95]. Por ejemplo, un estudio que investigó el impacto del ejercicio aeróbico en mujeres con sobrepeso durante un programa de seis semanas encontró correlaciones claras entre metabolitos en suero y heces, señalando una mayor oxidación de lípidos y estrés oxidativo [96]. En particular, se observó un aumento en los niveles de lisofosfatidilcolina en suero y glicerofosfocolina en heces, que se asoció con la abundancia de *Akkermansia* [96].

Otro estudio con ciclistas profesionales durante "La Vuelta" de 2019 analizó la dinámica de la microbiota intestinal y el contenido de SCFAs a lo largo de la competencia [97]. Los resultados mostraron que la composición de bacterias como *Bifidobacteriaceae, Coriobacteriaceae* y *Erysipelotrichaceae* tenían un valor predictivo alto sobre el rendimiento final de los atletas [97]. La relación bidireccional entre el ejercicio y el entorno microbiano también ha sido explorada en revisiones recientes, las cuales han destacado que los metabolitos producidos por las bacterias, como los SCFAs, pueden mejorar la eficiencia metabólica de los atletas [98]. Estas revisiones subrayan que tanto el ejercicio a largo plazo como los patrones dietéticos asociados a la práctica deportiva de alto nivel influyen en la composición del microbioma [98].

7. OBJETIVOS

OBJETIVO GENERAL

Evaluar la relación entre el microbioma intestinal y la actividad física en la literatura mediante revisiones sistemáticas y en humanos a través de una aproximación metagenómica y metabolómica.

OBJETIVOS ESPECÍFICOS

1. Describir la relación entre el nivel de actividad física y la microbiota intestinal en humanos mediante revisiones sistemáticas de la literatura en adultos aparentemente sanos y en adultos mayores.

2. Caracterizar y comparar el perfil taxonómico del microbioma intestinal de no deportistas y deportistas colombianos pertenecientes a las disciplinas de ciclismo de ruta y levantamiento de pesas.

3. Evaluar la abundancia de genes, rutas metabólicas y metabolitos clave en el microbioma intestinal de deportistas colombianos pertenecientes a las disciplinas de ciclismo de ruta y levantamiento de pesas, mediante una aproximación integrativa de metagenómica y metabolómica no dirigida.

8. INTRODUCCIÓN A LOS CAPÍTULOS

Los beneficios de la actividad física son ampliamente reconocidos y han sido documentados en numerosos estudios. La reducción del comportamiento sedentario y el aumento en los niveles de actividad física conducen a una serie de modificaciones tanto moleculares como estructurales en el organismo. A través de una revisión exhaustiva de la literatura, se identificó que las diversas metodologías empleadas para estudiar el microbioma intestinal, incluyendo enfoques ómicos, han revelado beneficios adicionales asociados con la práctica del ejercicio físico mediante la modificación de la microbiota intestinal. Sin embargo, es fundamental considerar varios factores en esta relación. Durante el desarrollo de esta tesis, se destacó el papel crucial que desempeñan el tipo y el nivel de entrenamiento. Se observó que los individuos con altos niveles de actividad física y un comportamiento sedentario reducido presentan una mayor abundancia de bacterias beneficiosas, como las pertenecientes a los géneros *Bifidobacterium*, *Akkermansia* y *Bacteroides*.

Además, las revisiones sistemáticas realizadas en el primer capítulo revelaron que los resultados no siempre son concluyentes. No se observaron consistentemente los mismos cambios o efectos relacionados con la práctica de actividad física en adultos y en adultos mayores. Esto nos llevó a reconocer la diversidad de factores tanto del hospedador (ser humano) como de las características del microbioma intestinal. Estos factores pueden incluir la ubicación geográfica, los hábitos alimenticios, la edad, el género y el índice de masa corporal, entre otros.

Uno de los vacíos de información más destacados que identificamos en este capítulo fue la escasez de estudios en Latinoamérica, particularmente en la población colombiana. Además, encontramos una falta de información relacionada con otras formas de entrenamiento, como el enfoque en la fuerza muscular en lugar de la resistencia cardiovascular, que ha sido el centro de la mayor parte de la evidencia científica. Si bien mejorar la capacidad cardiopulmonar y la resistencia cardiovascular confiere beneficios específicos al microbioma intestinal, como el aumento en la producción de ácidos grasos y una mejor utilización de sustratos provenientes de la dieta, no logramos identificar un efecto similar en actividades que requieren el uso de otros sistemas, como el muscular.

Basándonos en las razones expuestas anteriormente, la información recopilada durante la exhaustiva revisión de la literatura nos permitió diseñar un estudio de corte transversal con el objetivo de proporcionar datos sobre la población colombiana que participa en dos tipos específicos de entrenamiento: fuerza muscular y resistencia cardiovascular. En esta segunda parte de la tesis, también incorporamos un grupo de sujetos no deportistas para poder comparar la diversidad taxonómica del microbioma intestinal entre deportistas y no deportistas colombianos.

Esta sección de la tesis se divide en dos capítulos. En primer lugar, nos centramos en describir la comunidad de microorganismos presentes en deportistas colombianos que practican ciclismo de ruta y levantamiento de pesas. La selección de representantes de estos deportes se basó en la necesidad de comparar sujetos con diferentes procesos de adaptación al entrenamiento físico. Por lo tanto, reclutamos a individuos definidos como deportistas, quienes participaban en ligas y/o

federaciones colombianas, seguían un plan de entrenamiento específico para su deporte durante al menos dos años y se encontraban en fase precompetitiva y en concentración para una competencia nacional o internacional. Estas características se definieron con el objetivo de garantizar la homogeneidad de la muestra y asegurar que los sujetos fueran deportistas profesionales. Asimismo, la selección del grupo de no deportistas se realizó con la premisa de incluir sujetos que no practicaran ningún tipo de actividad física y que llevaran un estilo de vida predominantemente sedentario, pero que mantuvieran un estado aparentemente saludable y un índice de masa corporal (IMC) dentro de un rango saludable (18 – 24.9 kg/m^2). Esto se hizo con el fin de evitar confusiones con otros factores, como enfermedades cardiometabólicas o el consumo de cigarrillos, entre otros.

Para el tercer y último capítulo, se buscó integrar los resultados obtenidos de un estudio funcional del microbioma intestinal mediante un enfoque de integración ómico. Esto nos permitió obtener una amplia gama de información genética y metabólica, incluyendo genes y representaciones de reacciones enzimáticas (RXN). Además, se propuso un modelo de estudio de la interacción entre el microbioma y el huésped en sujetos altamente entrenados. Este modelo incluyó la integración de datos provenientes del metaboloma fecal, que refleja los subproductos del microbioma intestinal, así como datos del metaboloma sanguíneo y del lipidoma. Estos datos permitieron rastrear compuestos diferenciales entre los procesos de adaptación propios de las dos disciplinas estudiadas y ofrecieron posibles rutas de intercambio de información entre el microbioma y el hospedador.

Dado el carácter complejo de la relación entre el microbioma y el huésped en contextos de actividad física y deporte, así como la variedad de enfoques requeridos para comprender esta relación, esta tesis se propone abordar su objetivo general a través de tres capítulos distintos.

CAPÍTULO 1: Relación entre la microbiota intestinal y la actividad física en humanos; revisión sistemática de la literatura.

CAPÍTULO 2: Caracterización y comparación taxonómica del microbioma intestinal de deportistas y no deportistas colombianos.

CAPÍTULO 3: Interacción microbioma - hospedero en sujetos altamente entrenados: una aproximación desde las ciencias ómicas.

CAPÍTULO 1

El capítulo 1 tuvo como objetivo específico realizar dos revisiones sistemáticas que abordaran la relación entre la actividad física y el microbioma intestinal. Para ello, se siguieron las guías del manual de expectativas metodológicas de las revisiones Cochrane de intervención (MECIR) y la declaración PRISMA, asegurando la rigurosidad del proceso.

Las revisiones se enmarcaron en la metodología PICO/PECO, que permitió formular preguntas de investigación claras y específicas. Se segmentaron a los participantes por rangos de edad, enfocando la primera revisión en personas aparentemente sanas de 18 a 45 años y la segunda en adultos mayores de 65 años. Esta diferenciación fue fundamental, ya que facilitó un análisis más preciso y contextualizado de los efectos del ejercicio en el microbioma intestinal.

Para llevar a cabo las revisiones, se establecieron criterios siguiendo las pautas de PROSPERO y se utilizaron términos de búsqueda en inglés, conforme a los estándares de indexadores MeSH y EMTRE. Las bases de datos consultadas incluyeron Web of Science, NIH/PubMed, Ovid/Medline y Academic Search Complete. La calidad metodológica y el riesgo de sesgo de cada estudio se evaluaron mediante la herramienta ROBINS-I, garantizando la validez de los hallazgos.

Los resultados de estas revisiones revelan la diversidad de efectos que la actividad física puede tener sobre el microbioma intestinal, además de las diferencias significativas entre los grupos etarios analizados. A lo largo del capítulo, se discuten las implicaciones de estos hallazgos, así como las limitaciones identificadas en la literatura existente. Los detalles de los resultados pueden encontrarse en los artículos que se presentan a continuación.

- **Artículo 1: Aya V,** Flórez A, Perez L, Ramírez JD. Association between physical activity and changes in intestinal microbioma composition: A systematic review. PLOS ONE. 2021 Feb 25;16(2):e0247039.

- **Artículo 2: Aya V,** Jimenez P, Muñoz E, Ramírez JD. Effects of exercise and physical activity on gut microbioma composition and function in older adults: a systematic review. BMC Geriatr. 2023 Jun 12;23(1):364.

CAPÍTULO 1: Relación entre la microbiota intestinal y la actividad física en humanos; revisión sistemática de la literatura

PLOS ONE

RESEARCH ARTICLE

Association between physical activity and changes in intestinal microbiota composition: A systematic review

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Abstract

Introduction

The intestinal microbiota comprises bacteria, fungi, archaea, protists, helminths and viruses that symbiotically inhabit the digestive system. To date, research has provided limited data on the possible association between an active lifestyle and a healthy composition of human microbiota. This review was aimed to summarize the results of human studies comparing the microbiome of healthy individuals with different physical activity amounts.

Methods

We searched Medline/Ovid, NIH/PubMed, and Academic Search Complete between August–October 2020. Inclusion criteria comprised: (a) cross-sectional studies focused on comparing gut microbiome among subjects with different physical activity levels; (b) studies describing human gut microbiome responses to any type of exercise stimulus; (c) studies containing healthy adult women and men. We excluded studies containing diet modifications, probiotic or prebiotic consumption, as well as studies focused on diabetes, hypertension, cancer, hormonal dysfunction. Methodological quality and risk of bias for each study were assessed using the Risk Of Bias In Non-randomized Studies—of Interventions tool. The results from cross-sectional and longitudinal studies are shown independently.

Results

A total of 17 articles were eligible for inclusion: ten cross-sectional and seven longitudinal studies. Main outcomes vary significantly according to physical activity amounts in longitudinal studies. We identified discrete changes in diversity indexes and relative abundance of certain bacteria in active people.

Conclusion

As literature in this field is rapidly growing, it is important that studies incorporate diverse methods to evaluate other aspects related to active lifestyles such as sleep and dietary

Competing interests: The authors have declared that no competing interests exist.

patterns. Exploration of other groups such as viruses, archaea and parasites may lead to a better understanding of gut microbiota adaptation to physical activity and sports and its potentially beneficial effects on host metabolism and endurance.

Introduction

The intestinal microbiota comprises bacteria, fungi, archaea, protists, helminths and viruses that symbiotically inhabit the human digestive system, with five bacterium phyla—*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*—representing the predominant microorganisms in the gut [1, 2]. The term "microbiome" refers to the collective genome of these microbes [1, 2].

Despite coordinated efforts by several international consortia to define the composition of a healthy microbiota [3], the term currently remains incomplete given the many intrinsic and extrinsic factors associated with gut ecosystems [4–7].

Numerous studies have aimed to establish the role of environmental and behavioral factors on microbiota composition [8–14]. Diet is currently considered the main extrinsic factor [15, 16] followed by sleep, circadian rhythm [17], and physical activity [7]. Similarly, the presence and progression of disease might change the abundance and diversity of bacteria [4]: chronic illnesses such as irritable bowel syndrome (IBS) [8, 9], type 2 diabetes (T2D) [10], hypertension [11], and cancer [12] are associated with abnormal microbiota composition and function. Changes in microbiota diversity may diminish the abundance of beneficial bacteria while favoring the growth of potentially pathogenic microorganisms, a process known as "dysbiosis", which can further impact host metabolism [18, 19]. In obesity, for example, changes in the abundance of Bacteroides and Firmicutes (B/F ratio), may promote fat storage, increase energy collection from nutrients, and decrease energy expenditure [13]. The stability and diversity of microbiota also vary across age [14, 20] with larger microorganism diversity associated with adulthood with healthy habits [21, 22].

Active lifestyle behaviors improve several metabolic and inflammatory parameters in chronic diseases: exercise regimes have been used as therapeutic strategies against obesity and T2D [23]. Physical activity promotes adaptational changes on human metabolic capacities to reach a specific goal, which could be competitive in the case of athletes, or recreational and aesthetic in the non-competitive population. Diet is also a major target to meet these objectives as the consumption of dietary supplements is common in active people [24, 25] and probiotic consumption emerges as a profitable market due to its possible effects on gut epithelium homeostasis, especially for competing athletes [26]. However, this is a growing research field and there are still some concerns about its positive impact on human gut microbiota [27].

It is still unclear whether an active lifestyle, a healthy diet, or a combination of both can influence intestinal microbiota towards a healthy state. Animal models have allowed researchers to develop physiological and biochemical protocols [28–38] to explore the functional effects of exercise on the microbiome [37, 39, 40], and cross-sectional and longitudinal studies have tried to describe the effects of physical activity on the microbiome composition of active versus non-active adult humans. The heterogeneity of methodological approaches and the lack of standardized criteria for active/non-active people is one of the largest challenges in this research field.

This review aims to summarize the results of all human studies comparing the microbiome composition of healthy individuals with different physical activity amounts (PAA).

Methods

Reporting

Results from this study were reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines [31].

Search strategy

A computerized search was conducted between August–October 2020 using standardized English search terms assigned by the MeSH and EMTRE indexes using the Boolean operators OR/AND: "exercise" OR "physical activity" AND "human" AND "gastrointestinal microbiome" OR "gut microbiota". The databases consulted included Medline/Ovid, NIH/PubMed, and Academic Search Complete. The results from cross-sectional and longitudinal studies are shown independently.

Inclusion and exclusion criteria

We included the following research in our review: (a) cross-sectional studies focused on comparing gut microbiome among subjects with different physical activity levels—from athletes to inactive individuals—, using guidelines from the American College of Sports Medicine (ACSM) [41]; (b) studies describing human gut microbiome responses to any type of exercise stimulus; (c) studies containing healthy adult women and men (18–45 years old); (d) studies written in English.

We excluded studies containing diet modifications, probiotic or prebiotic consumption, as well as studies focused on diabetes, hypertension, cancer, hormonal dysfunction, or related illnesses since evidence suggests that these conditions may lead to significant changes in the composition of gut microbiota. Reviews, comments, letters, interviews, and book chapters were also excluded. PRISMA flow diagram (Fig 1) shows the screening process for this systematic review [42].

Quality assessment

Methodological quality and risk of bias for each study were assessed using the Risk Of Bias In Non-randomized Studies—of Interventions tool (ROBINS-I) [43]. This tool provides a detailed framework for assessing the risk of bias domains from Non-randomized studies of interventions (NRSIs).

Once a target trial specificity to the study was designed and confounding domains were listed, the risk of bias was assessed specifically for the comparisons of interest to this review. The overall risk of bias judgment can be found in S1 Table.

Supplementary document includes a checklist based on the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA).

Results

Literature search

654 articles were retrieved from the databases. Duplicate studies were identified and removed, leaving only 467 articles for screening. Once records were screened by title and abstract, a total of 359 articles were excluded. After careful reading of the methodology section of the remaining 108 potential eligible articles, exclusion criteria were applied.

Finally, a total of 17 studies were included in this review. Recorded outcome measures included differences for α and β diversity and relative abundance ($p < 0.05$). Transcriptional

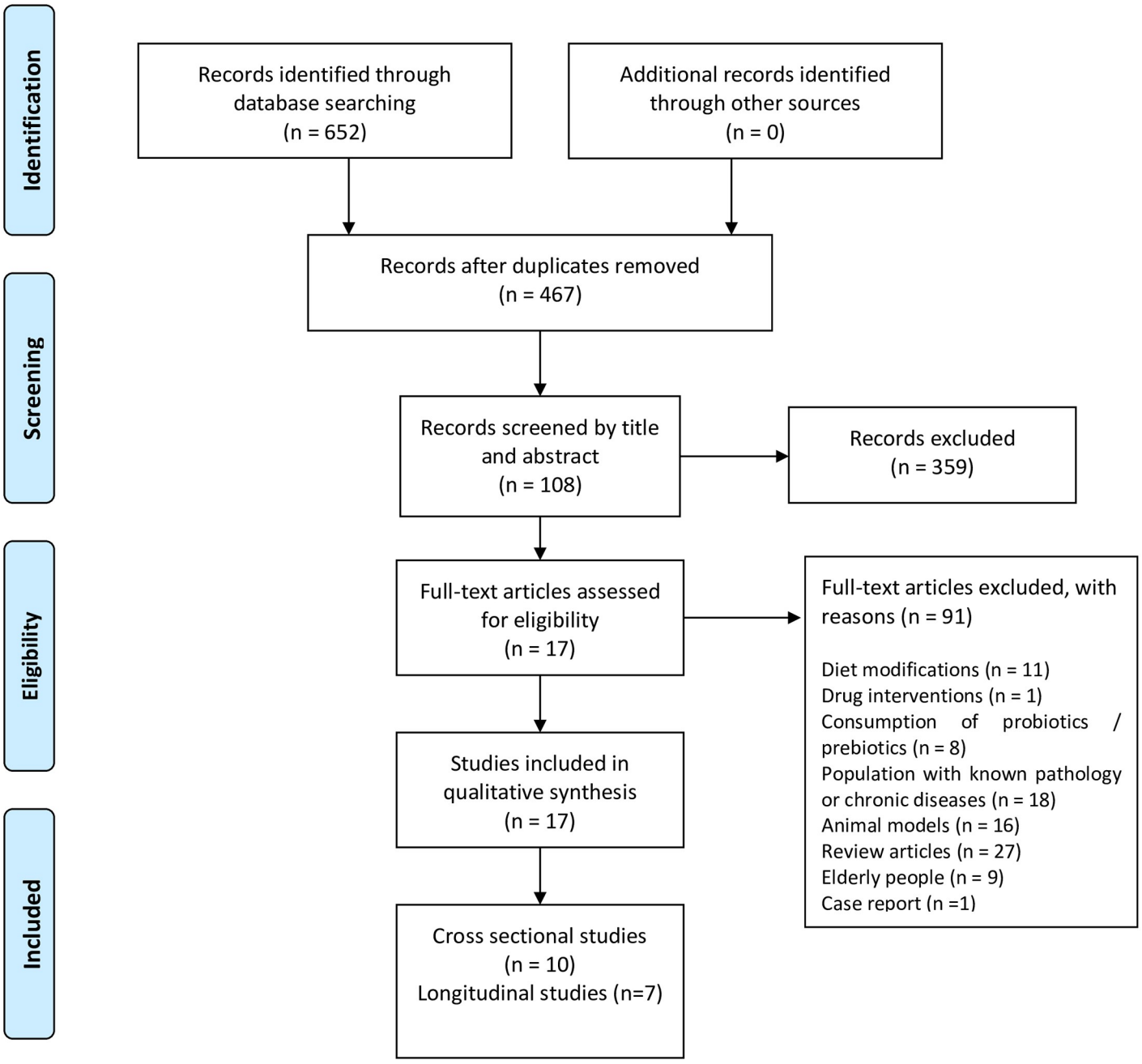

Fig 1. PRISMA flow diagram employed for this systematic review.

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and metabolomic data extracted from feces were included. Measurements or description of physical activity amounts (PAA) were used to classify results based on inactive, active, and athletic subjects.

Different levels of physical activity on gut microbiota

Table 1 shows a set of studies aiming to establish whether meeting the recommended physical activity quotas [41] influences microbiota composition measured as physical activity amounts (PAA) and abundance/diversity respectively.

Table 1. Summary of cross-sectional studies comparing intestinal microbiota between groups with different Physical Activity Amounts (PAA).

Reference	Publication year	N	Sample	Comparison axis	Results	Country
** Clarke et al. [44] Barton et al. [45]	2014	86	Rugby players (29 ± 4) and two control groups with different BMI (29 ±6)	Athletes and non-athletes with distinct BMI ≤25 –>28	Higher diversity in athletes (Shannon index, p = 0.0064) Relative abundance Athletes vs IMC ≤25 ↑ 40 taxa ↓ *Bacteriodetes* ↓ *Lactobacillaceae* ↓ *Lactobacillus* **Athletes vs IMC >28** ↑ 48 taxa ↑*Akkermansiaceae* (family) ↑*Akkermansiaceae* (genus) ↓*Bacteriodetes* (genus)	Ireland
Estaki et al. [46]	2016	39	Healthy young men and women (26.2 ± 5.5)	Categories of cardiorespiratory fitness: High–Avg–Low	No differences in α and β diversity	Canada
Bressa et al. [47]	2017	40	Middle-aged women (ACT 30.7 ± 5.9 –SED 32.2 ± 8.7)	Physical activity level and sedentary behavior	No differences for α and β diversity among groups **Sedentary women** ↑*Barnesiellaceae* (family & genus) ↑*Odoribacteraceae* (family & genus) ↑*Bifidobacterium* (genus) ↑*Turicibacter* (genus) ↑*Clostridiales* (genus), ↑*Coprococcus* (genus) ↑*Ruminococcus* (genus) **Women with an active lifestyle** ↑*Faecalibacterium prausnitzii* (spp.) ↑ *Roseburia hominis* (spp.) ↑ *Akkermansia muciniphila* (spp.)	Spain
Petersen et al. [48]	2017	71	Cyclists (women and men) with ≥2 years participating in competitive events	Performance level: professional vs amateurs	Higher diversity in cluster 3 (11 professional and 3 amateur cyclists) Shannon index p = 0.0004 Higher abundance of the genus: *Bacteroides*, *Prevotella*, *Eubacterium*, *Ruminococcus*, and *Akkermansia*	United States
Yang et al. [49]	2017	71	Premenopausal women age between 19 and 49 years	Cardiorespiratory fitness (CRF): High–Low	High CRF ↑*Bacteroides* ↓ *Eubacterium rectale*	Finland
Whisner et al. [50]	2018	82	University students (men and women) (18.4 ± 0.6)	Physical activity level and sedentary behavior	No differences in α and β diversity	United States
Durk et al. [51]	2019	38	Apparently healthy men and women (25.7 ± 2.2)	Comparison between gender and oxygen consumption (VO2peak)	No differences in α and β diversity	United States
Jang et al. [52]	2019	45	Bodybuilding (n = 15), athletes (n = 15), non-athlete control group (n = 15)	Differences among sporting activity	Relative abundance in bodybuilders ($p < 0.05$) ↑*Faecalibacterium* ↑*Sutterella* ↑*Clostridium* ↑*Haemophilus* ↑*Eisenbergiella* ↓*Bifidobacterium* ↓*Parasutterella* Athletes and control group ($p < 0.05$) ↑*Bifidobacterium adolescentis* ↑*Bifidobacterium longum* ↑*Lactobacillus sakei* ↑*Blautia wexlerae* ↑*Eubacterium hallii*	South Korea
O'Donovan et al. [53]	2019	37	International level athletes	Differences among sports classification groups	↑*Eubacterium rectale* ↑*Polynucleobacter necessarius* ↑*Faecalibacterium prausnitzii* ↑*Bacteroides vulgatus* ↑*Gordonibacter massiliensis*	Ireland

(*Continued*)

Table 1. (Continued)

Reference	Publication year	N	Sample	Comparison axis	Results	Country
Liang et al. [54]	2019	28	Wushu martial arts athletes (20.1 ± 1.8)	High (H) and Low (L) levels of competition	H group Higher α diversity (Shannon index p = 0.019 and Simpson diversity index p = 0.001) ↑*Parabacteroides*, ↑*Phascolarctobacterium* ↑*Oscillibacter* ↑*Bilophila* ↓*Megasphaera*	China

* Reported findings refer to the composition of the microbiota in terms of diversity (α and β) and species abundance where arrows denote increase (↑) or decrease (↓). Only significant results are shown ($p < 0.05$)

CRF = cardiorespiratory fitness

** Studies including same population.

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Notable differences have been described between competing athletes and inactive people: (a) greater microbiota α-diversity has been reported in athletes—highly associated with dietary patterns and protein consumption [44, 45]; and (b) a significative abundance of *Lachnospiraceae*, *Akkermansiaceae* and *Faecalibacterium* bacteria coupled with a lower abundance in *Bacteroidetes* phylum has been reported in active women [47].

Researchers have also considered associations between cardiorespiratory fitness (CRF) and the composition of gut microbiota, although no significant differences in α or β diversity indexes were reported between high, medium, and low VO2 consumption [46, 49, 51]. VO2peak was a significant predictor of α-diversity, with the Species Richness index significantly ($p = 0.011$) associated with increasing VO2peak (Radj2 = 0.204) [46]. It is important to note that no differences related to gender and oxygen consumption have been reported in the gut microbiota field [51].

Gut microbiota composition in studies involving athletes

We included studies that compared microbiota composition and diversity of individuals from different sporting disciplines [45, 48, 52–55]: (a) some disciplines were strongly associated with a relative abundance of bacteria as described in Table 1 [52]; (b) athletes from distinct disciplines and level of competition displayed significative differences in microbiota diversity and species richness [48, 54]; and (c) high-performing individuals have been reported with a greater abundance of the genera *Parabacteroides*, *Phascolarctobacterium*, *Oscillibacter*, *Bilophila* and a lower abundance of *Megasphaera* [54].

Characteristics related to training loads were also explored. A study positively correlated α and β-diversity with decreased training volume per week during a two-week follow-up on a group of swimmers [56]. In a different study, dynamic and static components of sports practice [57] were used to cluster a sample of Olympic athletes revealing no significant differences in diversity indexes in groups categorized by energy demand, with the whole sample exhibiting an abundance of the species *Eubacterium rectale*, *Polynucleobacter necessarius*, *Faecalibacterium prausnitzii*, *Bacteroides vulgatus* and *Gordonibacter massiliensis* [53].

Changes in composition and function of the intestinal microbiota after an exercise program and sporting events

Fig 2 shows how phenotypic characteristics of the host and stimulus exposure times can induce changes in specific groups of bacteria when starting an exercise program. Body mass index (BMI) appears to be a determining factor in microbiota response to exercise. Fecal microbiota

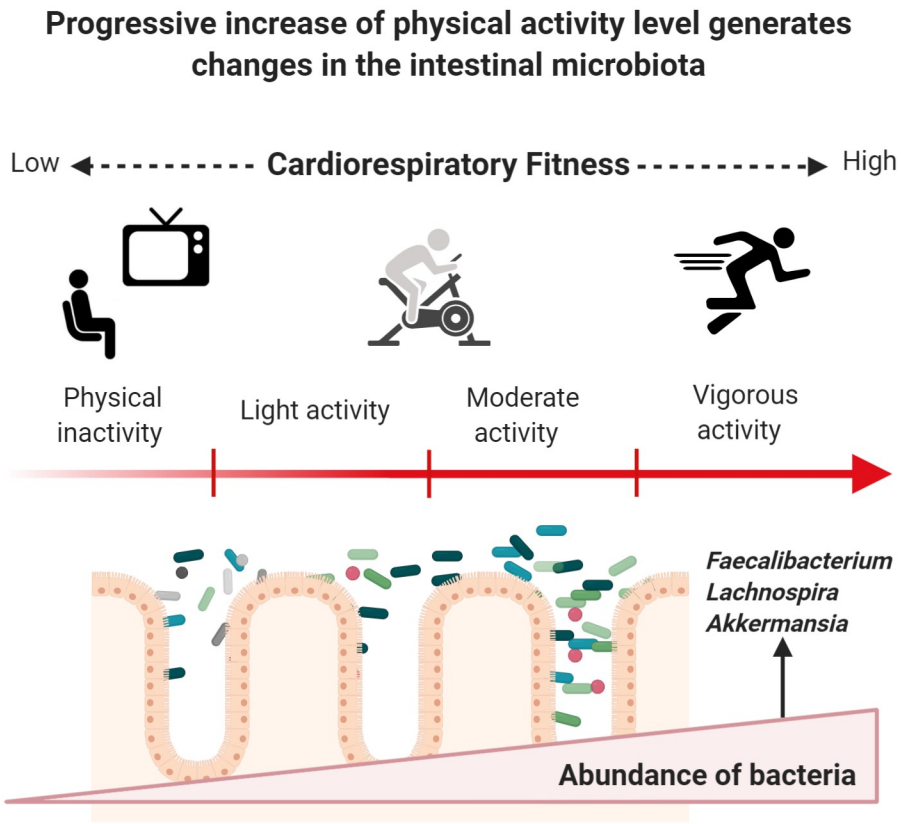

Fig 2. Exercise induces changes in gut microbiota through enhanced CRF in previously inactive subjects. Once a subject increases PAA, a series of beneficial molecular adaptations are induced allowing the enhancement of CRF. Major oxygen consumption is related to lower cardiometabolic risk, which may occur by progressively increasing energy-demanding activities based on endurance training. Physiological modifications occur, and the gut microbiota does not appear to play a role in this process. Recent research has provided insight into a progressive increase of helpful members from different phyla of bacteria. However, these changes could depend on BMI status, energy demand, and exposure time to exercise. Figure created with Biorender.com.

https://doi.org/10.1371/journal.pone.0247039.g002

from apparently healthy individuals with a BMI $\geq$ 25 kg/m^2 shows discrete incremental changes regarding relative abundance of *Actinobacteria*, *Bacteroides*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia* phyla after 6 weeks of supervised aerobic training (Table 2). Gut microbiota from lean subjects responds to aerobic exercise by increasing the abundance of species from *Faecalibacterium* spp. and *Lachnospira* spp., and by reducing *Bacteroides* members [58, 59]. The only randomized intervention study to date reported a significant difference in Shannon index values with higher diversity in groups after 3 and 6 months of vigorous-intensity physical activity (70% of peak VO2) in subjects aged between 20 and 40 years who were overweight or obese in comparison to control group. Significant reduction of fat mass and increased CRF were observed in both groups while dietary values remained similar before and after the intervention [60]. To date, only one study has reported microbiota findings related to high-intensity interval training (HIIT) [61]; the authors of this non-randomized trial observed an increased in the abundance of *Subdoligranulumwa* (genus) in lean men ($p = 0.0037$) after three weeks of cycloergometer workout.

To identify whether there are changes after an endurance event, two studies collected samples before and after both a half-marathon and a marathon. The athletes had similar characteristics in terms of body composition, training level, diet, and age. The most significant change

Table 2. Longitudinal studies containing two or more samples of feces, before and after exercise activities.

Reference	Study type**	Year	Sample	Observation/intervention time	Comparison axis	Findings*	Country
Allen et al. [58]	Intervention	2018	N = 32 Women and men with different BMI: Lean n = 18 ($\overline{X}$ 25 years old) Obese n = 14 ($\overline{X}$ 31 years old)	Six weeks of aerobic exercise, duration 30 to 60 min and moderate-high intensity (60–75% HR)	Before and after exercise intervention	LEAN ↓*Bacteroides* ↑*Faecalibacterium* spp. ↑*Lachnospira* spp. OBESE ↓*Faecalibacterium* spp. ↑*Bacteroides* ↑*Colinsella*	United States
Munukka et al. [59]	Intervention	2018	N = 17 Sedentary middle age women BMI >27.5 kg/m2.	Six weeks of aerobic exercise	Before and after exercise intervention	↑*Dorea* ↑*Anaerofilum* ↑*Akkermansia* ↓*Porphyromonadaceae* ↓*Odoribacter* ↓*Desulfovibrionaceae* ↓*Enterobacteriaceae*	Finland
Kern et al. [60]	Intervention	2020	N = 88 Overweight and obese people age between 20–45 years old	Six weeks of intervention with different types of activities CON: No exercise (n = 14) BIKE: Active transport (n = 19) MOD: leisure time exercise (n = 31) VIG: vigorous supervised exercise (n = 24)	Control group vs rest of groups	Three months intervention VIG α diversity p = 0.012 6 months intervention VIG α diversity p = 0.059	Denmark
Zhao et al. [62]	Observational	2018	N = 20 Amateur athletes ($\overline{X}$ 31 years old)	Gut microbiota before and after running a half marathon, distance → 21 km BEF: Feces analyzed before half marathon AFT: Feces analyzed after half marathon	Before and After half marathon	BEF: ↓*Bacteroides coprophilus* AFT: ↑*Pseudobutyrivibrio* ↑*Coprococcus_2* ↑*Mitsuokella*	China
Scheiman et al. [55]	Observational	2019	N = 15 Professional marathonists ($\overline{X}$ 27.4 years old)	Feces recollected during one week before and after the Boston marathon, distance → 42 km	Before and After marathon	↑ *Veillonella* genus	United States
Hampton-Marcell et al. [56]	Observational	2020	N = 13 University swimmers aged between 18 and 24 years old	Feces recollected in three temporal phases of two weeks	Between phases and volume training	No differences for α—β diversity and abundance	United States
Rettedal et al. [61]	Intervention	2020	N = 29 men aged 20–45 years old (n = 14 lean; n = 15 overweihtg)	Nine sessions of HIIT on non-consecutive days	Pre–post HIIT intervention	No differences for α—β diversity and abundance between samples or exercise intervention.	New Zealand

*The reported findings refer to the composition of the microbiome in terms of diversity (α and β) and species abundance. Arrows denote an increase (↑) or decrease (↓). Significant results are shown ($p < 0.05$).

** Types of studies included: Intervention: The selected sample carried out an exercise program for a certain period. Observational: The selected sample was followed before and after a sporting event. **HR** = heart rate; **HIIT** = high intensity interval training.

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in microbiome composition was relative abundance. In the case of amateur athletes who ran 21 km, the presence of *Pseudobutyrivibrio*, *Coprococcus*_2, *Collinsella*, and *Mitsuokella* were significantly greater at the end of the race [62].

Another study included repeated samples from professionals athletes 1 week before and after running the Boston Marathon [55]. The results revealed a significant increase in *Veillonella*, a Gram-negative, anaerobic bacteria commonly found in gut and oral microbiota—with unique physiology including the capacity to obtain energy through lactate fermentation and

their inability to utilize glucose [55]. Metagenomic sequencing revealed an overrepresentation in the methylmalonyl-CoA pathway [55]. Isolation and subsequent treatment in mice with the strain *Veillonella atypica* were carried out to test whether mice inoculated with this bacterium or with *Lactobacillus bulgaricus* (control) exhibited altered responses after endurance training. The results revealed that animals treated with *V*. *atypica* showed a significant reduction in post-training proinflammatory cytokine levels, as well as better performance. No changes in GLUT4 glucose transporters were observed. To determine whether the richness of *Veillonella* causes functional changes in the emission of messengers such as short-chain fatty acids (SCFA), propionate from three samples was directly extracted and quantified using mass spectrometry. The results revealed an increased abundance of *Veillonella* and an improvement in lactate pathways. However, subsequent tests in animal models failed to demonstrate that resulting lactate could cross the lumen barrier and impact other tissues, such as muscle, brain or liver. Still, it could cross the barrier to the intestinal lumen [55].

Physical activity substantially improves metabolite synthesis associated with intestinal microbiota

Exercise in physically inactive individuals produces changes in the composition of the microbiome and improves the synthesis of metabolites associated with the intestinal microbiota. Table 3 describes findings related to functional changes in gut microbiota, where diverse techniques and predictive approaches were used across studies; Allen et al. [58] used gas chromatography to quantify Short Chain Fatty Acids (SCFAs) from fecal samples of people who started a 6-week program of mostly aerobic exercise. Participants were differentiated by BMI (lean and obese). Only samples from lean subjects (BMI <25.0 kg/m^2) exhibited a significant increase in SCFAs concentration after the training period, whereas the concentrations of acetate, propionate, and butyrate in the obese group (BMI >30 kg/m^2) remained unchanged. The significant increases in butyrate-producing bacteria in lean people such as *Roseburia* spp., *Lachnospira* spp., *Lachnospriaceae*, *Clostridiales*, and *Faecalibacterium* (Fig 2) were positively correlated to changes in butyrate concentrations and butyryl-CoA: acetate CoA-transferase (BCoAT) genes.

Performing energy-demanding activities (e.g., a half marathon) has also been associated with modifications in metabolites related to gut microorganisms. One study using metabolomic analysis with liquid chromatography of samples before and after a half marathon event in Shanghai reported an increase in the concentration of approximately 40 metabolites, mainly organic acids [62]. Results also revealed a decrease in 19 compounds (fold change > 0). The metabolic routes with the greatest changes after the race were pentose phosphate more enriched with a value of $q = 0.0071$, while biosynthesis of phenylalanine, tyrosine, and tryptophan was highly decreased [62]. In a similar study, Scheiman and collaborators used metaomics analysis in order to elucidate the metabolic contribution of *Veillonella* species, findings provide and insight about the role of bacteria in the systemic degradation of lactic acid after a high performance activity [55].

Discussion

Several microorganisms found in the gastrointestinal system of active individuals and elite athletes classify as beneficial bacteria (Fig 3). Tables 1 and 2 show an increased abundance of butyrate-producing bacteria like *Eubacterium rectale* in competitive athletes, which predominantly uses dietary starch but can also utilize by-products of resistant starch (RS) degradation produced by other bacteria [63, 64]. However, a greater abundance of this species has also been found in obese people and is linked to inflammatory status and dysbiosis [65]. Table 3 shows

Table 3. Description of possible functional changes associated with gut microbiota in physical activity and sports activities.

Reference	Metabolic approach used across studies	Outcomes related to functional changes	Possible pathways related to gut microbiota function and composition
Barton et al. [45]	Metagenomic analysis of fecal samples from rugby players (n = 40) and controls (n = 46) Quantification of SCFAs levels in feces gas chromatography–mass spectrometry.	Athletes had highest mean abundance across 29 of the 34 metabolic pathways categories stablished by authors. Levels of SCFAs were significantly higher in the athlete's group: acetate ($p<0.001$), propionate ($p<0.001$), butyrate ($p<0.001$) and valerate ($p = 0.011$).	Carbohydrate degradation Production of secondary metabolites and cofactors Production of SCFAs
Estaki et al. [46]	Analysis of SCFAs from the feces by gas chromatography (N = 39 healthy young adults with different levels of CRF)	VO2peak was strongly correlated with butyric acid mainly across HI and AVG fitness participants. Propionic and acetic acid were inversely correlated to VO2peak and were represented across LOW fitness participants.	The abundance of key butyrate-producing members from *Clostridiales*, *Roseburia*, *Lachnospiraceae*, and *Erysipelotrichaceae* genera was associated with the production of butyric acid.
Bressa et al. [47]	Fecal enzymatic activity from feces was quantified using a semi quantitative method (active and sedentary middle age women, N = 40)	The activity of cysteine aminopeptidase in feces was significantly higher in the active group than in the sedentary group.	Cysteine aminopeptidase activity was negatively correlated with the presence of *Bacteroides*.
Petersen et al. [48]	Alignment of mWGS reads to the KEGG database (N = 33 cyclists)	*Prevotella* transcriptional activity was positively correlated to three KEGG pathways and negatively correlated to two amino acid metabolism pathways. Positive associations between *Methanobrevibacter* and methane metabolism were reported.	Upregulation of methane metabolism was related to citrate cycle, oxidative phosphorylation, and pyruvate metabolism. Similarly, pathways in SCFA production, propanoate metabolism and butanoate metabolism, were upregulated along with methane metabolism.
O' Donovan et al. [53]	Fecal samples underwent 1H-NMR and UPLC-MS analysis (Olympic athletes)	Pathways involved with folate and amino acid biosynthesis, besides pathways involved with flavin biosynthesis and fermentation of sugar alcohols were representative in Olympic athletes.	Authors do not report significant correlations between metabolites and any individual species or pathways.
Liang et al. [54]	The abundance of functional categories of 28 Wushu martial were predict using PICRUSt based on closed reference OTUs.	Microbial gene functions related to histidine metabolism, chloroalkane and chloroalkene degradation and carbohydrate metabolism, were higher in elite Wushu martial artists.	Authors do not report significant correlations between microbial gene functions and any individual species or pathways
Allen et al. [58]	Concentrations of SCFAs was determined by gas chromatography before and after 6 weeks of aerobic training protocol. Functional Gene Quantification was assessed by qPCR	Aerobic exercise increased fecal concentrations of acetate, propionate and butyrate. Abundance of the butyrate-regulating gene BCoAT and the propionate-regulating gene mmdA was also observed after six weeks of training protocol. Results were dependent on BMI.	As a result of exercise program abundance of bacterial genera *Roseburia* spp., *Lachnospira* spp., *Clostridiales* spp., *Faecalibacterium* spp., and *f Lachnospiraceae unclass*., positively correlated with changes in butyrate and abundance of functional genes (BCoAT) whereas changes in *Bacteroides* spp. and *Rikenella* spp. negatively correlated with changes in butyrate and/or BCoAT gene.
Munukka et al. [59]	Metagenome analysis was performed after exercise training in 17 sedentary middle age women.	The metagenomics assessment of the functional genes revealed no changes in the major pathways after the exercise period enrichment of pathways were not altered	N/A
Zhao et al. [62]	Metabolites concentration in feces was measured by LC-MS in amateur runners before and after a half marathon. KEGG ID were used for KEGG pathway enrichment analysis and PICRUSt analysis was used to predict COG functions.	Diverse metabolites from the pentose phosphate pathway, pyrimidine metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis were significatively enriched. Three metabolites in the pyrimidine metabolism pathway was decreased after running the half marathon.	Functions of gut microbiota that significantly changed after race: ↑Cell motility function ↓Energy production and conversion Pathways associated with cell motility: ↑ Flagellar assembly ↑ Bacterial chemotaxis ↑Oxidative phosphorylation related to Energy production and conversion function.

(*Continued*)

Table 3. (Continued)

Reference	Metabolic approach used across studies	Outcomes related to functional changes	Possible pathways related to gut microbiota function and composition
Scheiman et al. [55]	Meta omics analysis	Identification of *Veillonella* (genus) as a functional member of gut microbiome related to athletes.	After numerous analysis researchers concluded that the metabolic pathway *Veillonella* species utilize for lactate metabolism is enriched specially in sportive people. This symbiotic member of gut microbiota could metabolize the systemic lactate resulting from muscle activity during exercise.

Arrows denote an increase (↑) or decrease (↓). Significant results are shown ($p < 0.05$). **mWNGS** = whole metagenome shotgun; **KEGG** = Kyoto Encyclopedia of Genes and Genomes; **1H-NMR** = Proton nuclear magnetic resonance; **UPLC–MS** = Ultra performance liquid chromatography–mass spectrometry; **BMI** = Body mass index; **LC–MS** = Liquid chromatography–mass spectrometry; **COG** = Clusters of Orthologous Groups; **CRF** = Cardio respiratory fitness; **HI** = High; **AVG** = Average.

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an increased in butyrate concentration and butyrate producers in different studies, this short chain fatty acid is considered beneficial [66] to gut health since it is a major energy source for enterocytes and plays a key role in the maintenance of epithelial homeostasis [66].

Similar findings have been reported for *Akkermansia muciniphila*, an abundant intestinal bacterium from the *Verrucomicrobia* phylum that has been related to healthy intestinal microbiota due to its capacity to colonize the mucosal layer and improve host metabolic immune responses by increasing mucus thickness [67, 68]. *A. muciniphila* also plays a key role in metabolic activity, leading to the production of beneficial SCFAs for hosts and other microbiota members [67–69]. Another common bacterium with increased abundance in active individuals is *Faecalibacterium prausnitzii*. This *Firmicutes* member is associated with immunomodulatory properties, leading to reduced levels of IL-2, the production of Interferon-gamma, and increased secretion of the anti-inflammatory cytokine IL-10 [70]. Evidence suggests a link between *F. prausnitzii* depletion and the onset of inflammatory bowel disease and Crohn's disease. Additionally, several studies also suggest that *F. prausnitzii* produces butyrate [66, 71]. Recently, the relationship between depletion of this gut microbiota member and the presence of sarcopenia has been explored in a small groups of older adults [72], the shotgun metagenomic sequencing approach used for this study allowed to identify a depletion in microbial genes involved in diverse metabolic pathways mainly SCFA synthesis, carotenoid and isoflavone biotransformation and amino acid interconversion in sarcopenic adults when compared to non-sarcopenic counterparts [72]. Although it is not possible to determinate if these metabolic pathways are specifically related to the abundance of *F. prausnitzii* or another gut microbial member, it is of interest that numerous studies involving active people report an increase of this *Firmicutes* member, future studies in this field should aim to study the relationship between the metabolic function of *F. prausnitzii* and physical activity in different age groups [73, 74].

Another microorganism with notable abundance among sportspeople is *Eubacterium hallii*, a commensal bacterium species that contributes to the formation of butyrate from glucose fermentation [75]; *E. halli* has trophic interactions with other bacteria from the *Bifidobacterium* family, which is beneficial for host metabolism [75, 76] and is capable of metabolizing 3-hydroxypropionaldehyde—an important compound in reuterin system, which highlights the importance of this strict anaerobe since it might be capable to catalyze the transformation of dietary cancerogenic PhIP to non-carcenogenic PhIP-M1 and thus exhibiting a protective function in the colon [75, 77].

Another interesting member of gut microbiota reported in active subjects is *Gordonibacter massiliensis* [53], a Gram-positive, motile non-spore-forming and obligate anaerobic

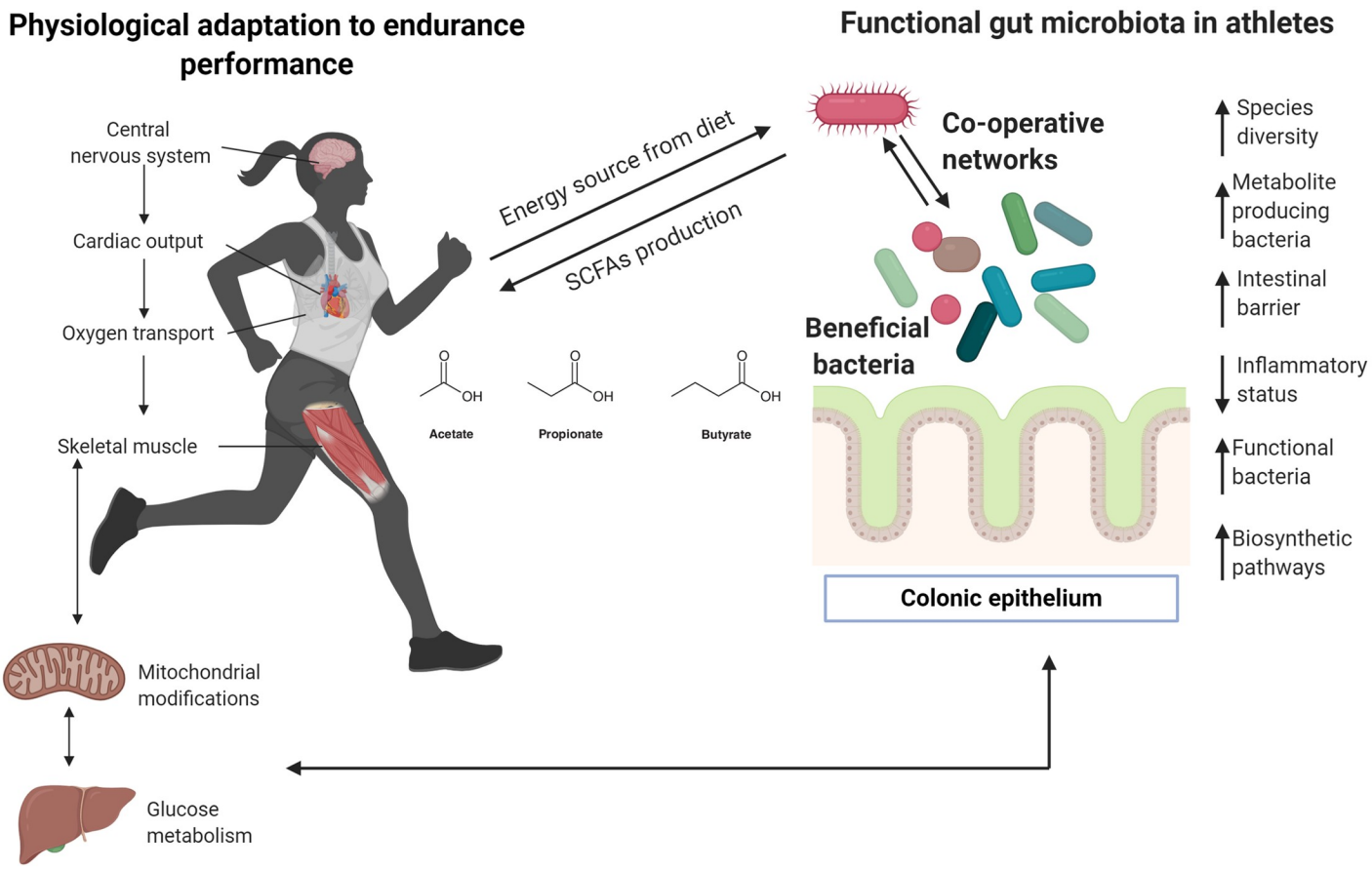

Fig 3. Insights about meta-community adaptation among gut microbiota in athletes. Most human physiological systems adapt to performance endurance, especially in elite and competitive athletes. Clear differences between physically inactive and athletic individuals have been described previously, particularly regarding cardiorespiratory adaptation given that endurance activities require major oxygen capacity to transport oxygen to different organs, including muscles and the liver. Efficient glucose and fatty acids metabolism are required to provide substrates that are finally transformed into energy by mitochondria. Recent research indicates that unique gut microbiota may be present in elite sportspeople, and special and unique bacteria can positively impact the host, providing substrates from the diet. Here, it is proposed that modifications of the gut microbiota ecosystem need to create co-operative networks to improve metabolic functions, particularly the production of biometabolites that can be used for the host (in this case, during highly demanding performance activities). Arrows denote an increase (↑) or decrease (↓). Figure created with Biorender.com.

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coccobacillus [78]. A similar member of the *Eggerthellacea* family (*G. urolithinfaciens)* can metabolize polyphenols from the diet into urolithin [79]. This bioavailable metabolite has been tested in cell lines and murine models as a new regulator of muscle trophism, as well as being implicated in androgenic pathways possibly via AMP-activated protein kinases to enhance (a) growth of myotubes and (b) protein synthesis with ulterior muscular hypertrophy [37]. Several biological activities have been related to urolithin B, including anti-inflammatory and antioxidant properties that play a protective role against neuroinflammation [44, 46]. It is not yet clear if *G. massilensis* can promote these effects via physical activity, and further research is needed to clarify this topic [80].

Some species detected in healthy and athletic humans are widely considered as candidate probiotics due to their beneficial effects. *Bifidobacterium adolescentis* exhibits strong resistant starch degrading activity [81]. *B. longum* has been extensively researched for its neuroenteric properties including stimulation of pathways between the gut and brain via the vagus nerve; normalization of anxiety-like behavior and hippocampal brain-derived neurotrophic factor in mice with infectious colitis; and modulation of resting neural activity, reduction of mental

fatigue, and improving activation of brain coping centers to counter-regulate negative emotions [82, 83]. *Roseburia hominis* is another flagellated gut anaerobic bacterium found in women with an active lifestyle and has been reported to exhibit immunomodulatory properties that could help treat gut inflammation, with beneficial properties for the intestinal barrier [84, 85]. *R. hominis* has been considered as a potential probiotic treatment [84, 85].

At the genus level, several species from *Parabacteroides* have been associated with the amelioration of obesity and the production of succinate and secondary bile acids [86, 87]. Among the *Firmicutes* phylum, the *Phascolarctobacterium* genus can produce SFCAs like acetate and propionate, and *Phascolarctobacterium* species have been reported in healthy and active individuals [88], with positive relationships with insulin sensitivity and secretion [89]. Although previous studies report diverse outcomes in subjects undertaking exercise programs, these differences could be attributed to differences in exposure times, which varied from 6-week to 6-month interventions. Besides, BMI and physical inactivity could be determinant factors for gut microbiota responses to exercise. The main type of exercise used in research thus far is endurance training (aerobic), in which the oxidative system is the most important pathway for energy production. Once a physically inactive subject initiates an aerobic training program, it typically takes between 4 to 6 weeks to exhibit acute cardiovascular adaptation to exercise depending on cardiac output and the capacity of active muscle to extract oxygen from arterial blood [90, 91]. Fig 2 represents a possible progressive effect exerted by exercise on gut microbiota once a physically inactive subject begins endurance training and enhances their CRF.

Exercise could also contribute to communication pathways between microbiota members, which constitute metacommunities that need to function in symbiotic environments (Table 3). The outcomes analyzed in this review suggest the importance of symbiotic bacteria, which can influence and enhance the function of other members of the gut ecosystem. The application of omics approaches may be crucial to identify other microorganisms with potential functional impact, as well as to verify possible relationships with systems and organelles proposed in past reviews [92]—especially with the mitochondria [93]. Notwithstanding the exploration of axes between gut microbiota and organs are beyond the scope of this review, studies included in this section allow identifying a relationship between gut, muscle, and brain that could explain a beneficial role physical activity on gut microbiota. This hypothesis has been proposed in past reviews where authors aim to explore physical activity as a key factor between neurodegenerative diseases and gut microbiota [94], and exercise as a modulator of intestinal microbiome [95]. Since it is not possible to draw firm conclusions about these interactions it is important to highlight the need to explore different sequencing methods and the incorporation of multi omics analysis to understand the metabolic adaptation of gut microbiota to exercise. Thus, some determinant factors such as training level, intensity and frequency of exercise might be relevant to elucidate the adaptative response of gut microbiome to exercise [95], therefore they must be taken into account in future studies.

Athletes typically display exceptional CRF [45, 96]. VO2max in competitive athletes can be up to twice as high as that of sedentary subjects, leading to a higher capacity for oxidative metabolism in muscles, better neural connections, and improved general metabolism [17] (Fig 3 and Table 3). The capacity to perform endurance activities, such as a marathon, depends on many factors, particularly muscle-buffering capacity and lactate metabolism [96]. Findings from longitudinal studies may help to understand the link between athletic gut microbiota and high-performance activities. Identification and isolation of *Veillonella* in endurance athletes suggests the communication between gut microbiota and muscle mediated by physical activity and the potential impact of anaerobic bacteria on propionate production via the Cori cycle should be further examined [55]. As shown in Tables 1 and 2, descriptive studies on the microbiome composition of high-performance athletes are limited. Although some relationships

between competition level and diversity/abundance of taxa have been established [48], it is important to examine information relevant to the phenotype as well as physiological adaptations which can vary substantially from one sports discipline to another.

Diet composition should not be overlooked and more detailed information should be gathered since the use of food frequency questionnaires gives a qualitative overview of food ingestion [97]. The use of dietary assessments methods like food diaries [98] can aid the quantitative and qualitative exploration of macro and micronutrients consumption [99]; it would also be relevant to determine the preliminary use of probiotics and prebiotics since its consumption has become recurrent in athletic population especially encouraged by the reported regenerative and immunologic benefits of *Lactobacillus*, *Bifidobacterium*, and *Bacillus* genera [26].

Limitations

This review attempts to summarize the main outcomes related to diversity and relative abundance across human studies involving different PAA. Although numerous studies have provided approaches for elucidating possible mechanisms, the effects of individual characteristics and the numerous factors that can influence the composition of the microbiome are important to consider. All studies included in this review were performed in relatively developed high-income countries. Although some reports have included the Hispanic population [50, 51], there is an important data gap from developing regions, particularly South America and Africa, which display substantial variations in the diet [100]. Similarly, the measurement of inflammatory and metabolic markers could provide useful information regarding the metabolism and physical condition of the host. The consumption of supplements and ergogenic aids from athletes should also be described in this field.

We also suggest that future randomized and non-randomized studies should try to implement direct methods like accelerometry to measure variables related to physical activity instead of auto-reported methods. Similarly, direct oxygen consumption measures, energy expenditure, and heart rate variability could help elucidate the systematic response of gut microbiota to exercise.

Conclusions

Our review aims to explore the possibility that exercise promotes the abundance of intestinal bacteria that could drive beneficial metabolic changes to human hosts (Figs 2 and 3). Further research is needed in the form of controlled clinical trials including different types of exercise (i.e. endurance and high-intensity training), distinct age groups, larger samples and incorporation of multi-omics approaches, as well as detailed information about diet and others lifestyle factors like sleep and circadian rhythm.

Few studies have expanded to other types of microorganisms, such as viruses [101] and archaea [48], while the role of parasites and fungi has not yet been identified. Exploration of other groups may help to comprehend the role of the gut microbiome on exercise adaptation, particularly muscle growth and biochemical signaling. One recent study reported the use of adeno-associated virus 9 (AAV9) as a vector to deliver the protein follistatin to improve muscle performance and mitigate the severity of osteoarthritis sequelae, including inflammation and obesity in mice [102]. Although this is a gene therapy approach, the possibility that viruses could mediate signaling pathways in short-to-long-term adaptation to exercise should not be neglected. The meta-community in the intestinal environment emphasizes the potential connections between bacteria, fungi, parasites, and viruses, which could directly influence the response to exercise [103, 104].

To date, it is not yet possible to draw firm conclusions about whether the metabolic activity of bacteria can impact various tissues via metabolites production during exercise in healthy humans. Nevertheless, the results reported by Scheiman et al. [55] appear to be significant, identifying a stable relationship between possible efficient microbiota members and the production of propionate, impacting lactate production, at least in the colonic lumen.

Future research examining the microbiota-exercise association should also aim to describe aspects of the lifestyle as well as possible, including diet, the level of training, and sedentary behavior.

Supporting information

S1 Checklist. PRISMA 2009 checklist.
(DOC)

S1 Table. ROBINS-I risk of bias assessment summary: Review authors' judgements about each methodological quality item for each included study in this review.
(DOCX)

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RESEARCH

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Effects of exercise and physical activity on gut microbiota composition and function in older adults: a systematic review

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Abstract

Background The characterization and research around the gut microbiome in older people emphasize microbial populations change considerably by losing the diversity of species. Then, this review aims to determine if there is any effect on the gut microbiota of adults older than 65 that starts an exercise intervention or improves physical activity level. Also, this review describes the changes in composition, diversity, and function of the gut microbiota of older subjects that had improved their physical activity level.

Methods The type of studies included in this review were studies describing human gut microbiota responses to any exercise stimulus; cross-sectional studies focused on comparing gut microbiota in older adults with different physical activity levels—from athletes to inactive individuals; studies containing older people (women and men), and studies written in English. This review's primary outcomes of interest were gut microbiota abundance and diversity.

Results Twelve cross-sectional studies and three randomized controlled trials were examined. Independently of the type of study, diversity metrics from Alpha and Beta diversity remained without changes in almost all the studies. Likewise, cross-sectional studies do not reflect significant changes in gut microbiota diversity; no significant differences were detected among diverse groups in the relative abundances of the major phyla or alpha diversity measures. Otherwise, relative abundance analysis showed a significant change in older adults who conducted an exercise program for five weeks or more at the genus level.

Conclusions Here, we did not identify significant shifts in diversity metrics; only one study reported a significant difference in Alpha diversity from overweight people with higher physical activity levels. The abundance of some bacteria is higher in aged people, after an exercise program, or in comparison with control groups, especially at the genus and species levels. There needs to be more information related to function and metabolic pathways that can be crucial to understand the effect of exercise and physical activity in older adults.

Trial registration PROSPERO ID: CRD42022331551.

Keywords Elderly, Gut microbiota, Gut microbiome, Physical activity, Exercise

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Background

According to the World Health Organization (WHO), the population aged 60 years will double by 2050, and it is projected that people older than 80 will triplicate. These increasing numbers could reach over two billion older adults in the following decades, becoming a significant health issue worldwide to ensure wellness [1].

The aging process is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death [2]. This natural condition affects most living organisms because of the decline of functionality as aging progress, conducted by cellular damage [3]. This deterioration has been widely studied in humans because it is the primary risk factor for significant pathologies [4]. Lopez-Otín and colleagues 2013 enumerated nine candidate hallmarks that represent common aging denominators and contribute to determining the aging phenotype [2]. However, recent studies of aging have planned new hallmarks compromising inflammation and microbiome disturbance, among others [5]. This new perspective could better explain health outcomes related to aging diseases and therapeutic studies to achieve a high-quality lifestyle for older people.

Current advances in sequencing technologies and bioinformatics pipelines have identified notable changes in the gut microbiota/microbiome through the lifespan and its substantial effects on human health [6]. The microbiome refers to the combined genetic material of all the microorganisms (bacteria, fungi, protozoa, megafauna, and viruses) living in a particular environment; this term is explicitly used to denote the genetic and functional diversity of the microorganisms community and its relationship with the host [7, 8].

In brief, the diversity and abundance of taxa that make up the gut microbiota (refers to composition) are highly susceptible to change. This is because of external and internal factors that are inherent to the human being, such as birth mode [9], presence or absence of diseases [10], geographical location [11], and diet [12], among others. Previous research shows that the intestinal microbiota in healthy individuals is stable, especially when there is an absence of clinical manipulation (for example, indiscriminate use of antibiotics) and healthy lifestyle habits, such as an adequate diet and moderate to vigorous physical activity [13]. An adequate balance of bacteria in the digestive tract ensures the microbiota works in a symbiotic environment with the host, however, changes in diversity could lead to a reduction in the abundance of beneficial bacteria and an increase in the prevalence of potentially pathogenic microorganisms, also called dysbiosis [14–17].

Diet is one of the most relevant environmental factors in the investigation of the intestinal microbiome since it modulates the population of microorganisms considerably [18], factors related to diet and nutrition status are key to modulating the composition of microorganisms that inhabit throughout the digestive system [19].

The relationship between the consumption of microbiota-accessible carbohydrates (MACs) and the production of butyrate, as well as the abundance of bacteria that produce this short-chain fatty acid, has been explored in human studies [20]. Significant reductions in the consumption of this macronutrient lead to a drastic decrease in *Bifidobacterium spp*., *Roseburia spp*., and *Eubacterium rectale*. Other microbiota members like *Clostridium spp*. are important for colon cells since they release butyrate as a final product of fermentation. However, the consumption of various starches and fibers can define the type of bacteria that abounds or impacts the intestine [21]. Also, the breaking of large chains of amino acids results in the generation of metabolites such as hydrogen, methane, carbon dioxide, some SCFAs, and branched-chain amino acids (BCAAs). These metabolites resulting from the fermentation of amino acids fulfill a wide range of biological functions for the host; however, the abundance of some of these compounds may be related to inflammation processes or chronic diseases, since large amounts can be detrimental to the intestinal environment [22].

Although it is not clear the underlying mechanisms that drive changes in the gastrointestinal microbiota under exercise conditions, a few studies involving omics sciences provide possible pathways [23–25]. Scheinman et al. identified in a cohort of athletes that the genus *Veillonella* increased considerably after running a marathon. Subsequent analysis of the *V. atypica* strain led the authors to conclude that this microorganism promotes an improvement in race time because of its conversion metabolism of exercise-induced lactate into propionate, thus identifying a natural enzymatic process encoded in the microbiome that enhances athletic performance through the Cori cycle [24]. One of the most relevant results is how intestinal colonization of *Veillonella* increases the Cori cycle by providing an alternative method of lactate processing whereby systemic lactate is converted into SCFAs that re-enter the circulation. SCFAs are absorbed in the sigmoid and rectal region of the colon and enter the circulation through the pelvic plexus, bypassing the liver and draining through the vena cava to reach the systemic circulation directly [24]. Microbiome-derived SCFAs then directly and acutely enhance performance, suggesting that the microbiome might access lactate generated during periods of sustained exercise and convert it into these athletic performance-enhancing SCFAs.

From infancy to old age, the gut microbiome follows some patterns related to rapid change, becoming

increasingly unique to individuals as they grow [26]. The characterization and research around the gut microbiome in older adults emphasize microbial populations change considerably by losing the diversity of species [27]; indeed, disturbances and diseases have been linked to these shifts [28, 29].

Recently, three independent cohorts comprising over 9000 individuals aged 18–87 characterized gut microbial patterns associated with age. They performed diversity analysis from multiple samples, paying particular attention to older adults. The results showed amplicon sequence variance (ASV) levels had a unique gut microbiome signature independent of sex or body max index and more related to age [26]. Otherwise, individuals over 80 exhibit continued microbial drift depending on health status. Wilmanski et al., identified microbiome patterns of healthy aging, such as depletion of core genera, primarily *Bacteroides* [26], and different microbial metabolic outputs in the blood, such as lower LDL cholesterol levels, higher levels of vitamin D and beneficial blood metabolites produced by gut microbes. These results are consistent with recent findings showing that host metabolism is crucial to understand the crosstalk between gut communities and the therapeutic alternatives [29, 30]. Despite the diet (a central shifter of the gut community [31, 32]), physical activity status is now considered a relevant factor in the study of the gut microbiome [33].

Physical activity (PA) is any movement produced by skeletal muscles that requires energy expenditure. The WHO includes leisure time, transport to and from places, and workdays as PA [34, 35]. The amount of activity can be quantified between low and vigorous intensity. Some types include walking, cycling, sports, and recreational activities [35], known to prevent and manage chronic non-transmissible diseases (stroke, diabetes, several cancers), many of which appear with aging [36].

PA and exercise training are well-known modifiable factors in aging, either for preventive medicine or chronic disease management. The protective effect and physiological response to exercise training have been extensively described [37, 38]: enhance the antioxidant response, promote activation of anabolic and mitochondrial biogenesis pathways in skeletal muscle [39], decrease inflammatory profile [40], improve insulin sensitivity, myokine profile and endothelial function [41, 42]. These changes confer multiple health outcomes, such as reducing symptoms of anxiety and depression [43], preventing falls and related injuries [41], improving all-cause mortality, an incident of type 2 Diabetes (T2D), specific cancers, or hypertension, and bone and muscular health. Physical exercise is associated with healthy aging, multisystemic benefits provided to this population are condensed in a multidimensional beneficial system; increased muscle synthesis, improved respiratory function, decreased blood pressure levels, and increased neurogenesis, as well as increased bone density muscle mass and loss of body fat percentage [44–46].

Several investigations have repeatedly shown that exposure to regular physical activity confers multiple positive effects on the aging process. The benefits of structured aerobic exercise programs are linked to better learning and cognitive performance on executive function and attentional control in aging [47, 48]. Bouts of physical activity also have a potential therapeutic capacity in conditions related to older adults, such as dementia [49]. Likewise, sufficient results from human and animal trials show the downregulation of pro-inflammatory cytokines and compounds by cardiovascular exercise [47, 50–52]. However, the effect of PA and exercise training on the composition and function of the gut microbiota in older people is not clear, considering the relevant role of the gut commensals for health outcomes and the modifications that confer augmenting PA [53–57].

Cross-sectional [54, 58, 59] and longitudinal [60–62] studies have sought to establish differences in the composition of the human gut microbiota related to physical activity level (PAL); however, the results are highly variable and sometimes contradictory. Only a few results suggest a significant difference in α and β diversity indicators between subjects with high and low PAL [54, 63]; meanwhile, other results show no change in the composition of gut microbiota related to exercise regimen [60, 64]. Modification of single bacteria taxa has been related to exercise stimulus, especially the increased abundance of *Lactobacillus, Bifidobacterium*, and *Akkermansia* [65]. Deeper analysis, specifically metabolome and metagenomic assays, shows significant changes in volatile compounds such as SCFAs [23] and unique members of the microbiome like *Veillonella* [24].

Studies seeking a link between physical activity and the gut microbiota include diverse age groups, such as older people [66–70] young adults [71, 72], adolescents [73], and mostly middle-aged women and men [54, 60, 61, 64, 74, 75]; likewise, diverse frequency, intensity, and type of exercise interventions can be found in these studies [24, 60, 76]. The growing evidence of the modulator effect of physical activity on the gut microbiota makes it relevant to conduct different systematic reviews where the type of population, type of studies, and type of exercise intervention are described.

Therefore, this systematic review aims to identify with the current and evidence whether starting an exercise program or improving PA level brings any notable change in the gut microbiota of adults older than 65 and whether these modifications are reflected in other physiological systems. This systematic review describes the changes

in composition, diversity, and function of the gut microbiota of older adults that have improved their physical activity levels.

Methods

Criteria for considering studies for this review

Types of studies

Since the gut microbiota research field in physical activity and exercise is growing, past reviews have showed that randomized control studies are few [53, 77]. For that reason, we consider involving: (a) studies describing human gut microbiota responses to any exercise stimulus (b) cross-sectional studies focused on comparing gut microbiota among older adults with different physical activity levels—from athletes to inactive individuals; (c) studies containing older adults women and men (+65 years old); (d) studies written in English. We excluded studies containing probiotic or prebiotic consumption and studies focused on diabetes and cancer. Reviews, comments, letters, interviews, and book chapters were also excluded. PRISMA Flow Diagram (Fig. 1) shows the screening process for this systematic review [78].

Types of participants

Populations studied in this review were women and men in older adults, which means over 65 years old. Since it is challenging to reach the elderly with no disease or medical condition, we defined our population as aged functional subjects with no physical limitation or physical disability. Studies involving people aged 65 years and older with only two medical conditions related to older adults or healthy were included.

Types of interventions

The focus of this review is to determine if starting any exercise intervention could significantly change the gut microbiota; for that purpose, we have established the following eligibility criteria for types of intervention a) randomized controlled trials designed to improve any of the muscular strength, endurance, or flexibility components of fitness in the population named before and b) non-randomized controlled trials designed to improve physical activity level through lifestyle interventions, cross-sectional studies will also be included.

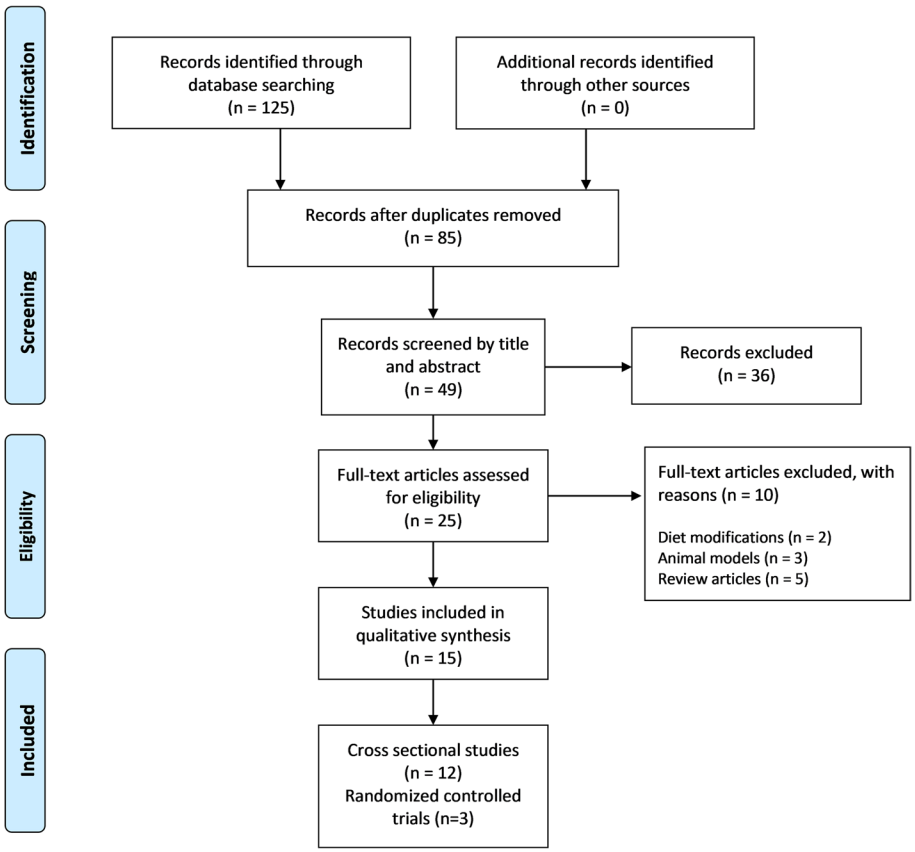

Fig. 1 Preferred Reporting Items for Systematic Reviews [78]

Types of outcome measures

The primary outcomes of interest are those related to the diversity and abundance of the gut microbiota. Secondary outcomes will focus on measures or approaches to the function of the gut microbiome. Also, quantification of physical activity level (E.g., median daily step counts) and outcomes related to maximum oxygen consumption and muscular strength will be considered.

The outcomes of interest for this review are:

-Gut Microbiota Abundance: one term frequently used in gut microbiota research is absolute abundance, which refers to the "unobservable actual abundance of a taxon in a unit volume of an ecosystem, such as the gut" [79]. It is essential to highlight that absolute and relative abundance are entirely different terms, according to Lin & Peddada. Changes in the absolute abundance of a single taxon can alter the relative abundance of all taxa [80].

These parameters are determined by the data got in the sequencing process; the next-generation sequencing (NGS) of the 16S rRNA helps describe microbial compositions in a niche. After a quality process, the 16S amplicon sequences can be clustered into Operational Taxonomic Units (OTUs) and Sequence Variants (SVs). In brief, observed counts of OTUs or SVs represent observed abundances of taxa in the sample [79–81].

-Gut Microbiota Diversity: Gut microbiota diversity refers to the number of different species present in a sample, niche, or ecosystem [82]. This review will be focused on stool samples provided by older adults involved in the studies that accomplished the criteria for inclusion. The microbial community in this niche has mainly been characterized in the past years [18]. The bacterial diversity defined by the numerical composition can be calculated with different indexes to determine the changes in the number of species [83]; Alpha diversity refers to the observed richness (number of taxa) and the relative abundances of those taxa (also known as evenness) within a sample. Meanwhile, Beta-diversity is defined as the variability in the microbial community composition among samples [84, 85].

Search methods for identification of studies

The search strategy is summarized in Table 1. The search terms "Elderly AND Gut Microbiota OR Gut Microbiome AND Physical Activity" were used in the bibliographic databases MEDLINE/Ovid, NIH/PubMed, and Academic Search Complete. This electronic search was done between May 14 and June 15, 2022, and other resources were not identified.

Quality assessment

Methodological quality and risk of bias for each study were assessed using the Risk Of Bias In Non-randomized Studies—of Interventions tool (ROBINS-I) [86] and the revised tool to assess the risk of bias in randomized trials (RoB 2) [87, 88].

Once a target trial specificity to the study was designed and confounding domains were listed, the risk of bias was explicitly assessed for the comparisons of interest to this review. The overall risk of biased judgment can be found in Supplementary Table 1 and Supplementary Table 2.

Results

Description of studies

After the electronic screening and evaluation of the preselected studies, we finally included fifteen studies in this review (Fig. 1). The type of study is significant cross-sectional, followed by controlled trials (randomized and non-randomized) and follow-up cohorts that were also included [89]. Table 2 collects relevant information from studies, such as medical conditions, age, and the number of participants who concluded the interventions and/or observations.

Results of the search

Likewise, cross-sectional studies did not reflect significant changes in gut microbiota diversity. No significant differences were detected among diverse groups in the relative abundances of the major phyla or alpha diversity measures (Chao 1, Simpson, Shannon; Kruskal–Wallis H test) [70, 89–96].

Otherwise, relative abundance analysis showed a significant change at the genus level in older adults who conducted an exercise program for five weeks or more. The relative abundance of *Clostridioides difficile* was

Table 1 Search strategy of the systematic review

Database	Search Query
Medline/Ovid	(elderly and (gut microbiota or gut microbiome)).ab. and (physical activity or exercise).ti
NIH/Pubmed	(((elderly [Title/Abstract]) AND (gut microbiota [Title/Abstract])) OR (gut microbiome [Title/Abstract])) AND (physical activity [Title/Abstract])
Academic Search Complete	elderly AND physical activity OR exercise AND gut microbiota

Table 2 Synopsis of the studies included

Reference	Year	Title	Country	Type of Study	N		Age		Medical condition
					♀	♂	Younger	Older	
[49]	2018	Effects of short-term endurance exercise on gut microbiota in elderly men	Japan	Randomized crossover trial		31	62	76	Arterial Hypertension. Dyslipidemia, Hyperglycemia, Prostatic Hyperplasia
[50]	2018	Gut dysbiosis is associated with the reduced exercise capacity of elderly patients with hypertension	China	Cross-sectional	24	32	65	80	Primary Hypertension
[51]	2019	The Association between Objectively Measured Physical Activity and the Gut Microbiome among Older Community-Dwelling Men	United States of America (metropolitan areas)	Cross-sectional		373	78	98	Osteoporotic Fractures
[52]	2019	Aerobic Exercise Training with Brisk Walking Increases Intestinal Bacteroides in Healthy Elderly Women	Japan	12-week non-randomized, comparative trial, where the allocation of the participants to either of the two exercise groups, AE and TM, was based on their preference	29		66	75	Healthy Sedentary Women
[53]	2019	Muscle strength is increased in mice colonized with microbiota from high-functioning older adults	USA	Cross-sectional/Experimental	13	16	70	85	Sedentary older adults, defined as the absence of structured exercise during the previous six months
[54]	2020	Physical fitness in community-dwelling older adults is linked to dietary intake, gut microbiota, and metabolomic signatures	Denmark	Cross-sectional	98	109	65	70	N/A
[55]	2020	Differences in Gut Microbiome Composition between Senior Orienteering Athletes and Community-Dwelling Older Adults	Ireland	Cross-sectional	51	45	68	76	N/A
[56]	2020	Effects of exercise frequency on the gut microbiota in elderly individuals	USA	Data available from American Gut Project (AGP) [57]	897		Normoweight = 462	Overweight = 413	Overweight and Obesity
[54]	2021	The Influence of Different Physical Activity Behaviours on the Gut Microbiota of Older Irish Adults	Ireland	Cross-sectional	100	54	56	69	Cardio Vascular Disease, Type 2 Diabetes Mellitus 7%

Table 2 (continued)

Reference	Year	Title	Country	Type of Study	N		Age		Medical condition
					♀	♂	Younger	Older	
[48]	2021	Strenuous Physical Training, Physical Fitness, Body Composition, and *Bacteroides* to *Prevotella* Ratio in the Gut of Elderly Athletes	Slovakia	A cohort of two years (follow-up)	22		63	67	N/A
[58]	2022	Increased physical activity improves gut microbiota composition and reduces short-chain fatty acid concentrations in older adults with insomnia	Israel	Cross-sectional	39	10	LOW 73.66 ± 6.65	HIGH 72.22 ± 5.08	Insomnia
[59]	2020	Effect of an 8-week Exercise Training on Gut Microbiota in Physically Inactive Older Women	China	Randomized controlled trial	6	6	60	75	HbA1c < 6.5%; (3) fasting blood glucose < 7.0 mmol/L; (4) ability to live independently in the community without restrictions on gait or balance; and (5) no diagnosis of type 1 diabetes mellitus or type Two diabetes mellitus
[15]	2021	Effect on the gut microbiota of 1-y lifestyle intervention with the Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus Study	Spain	1-year lifestyle intervention	183	179	55	75	HTA
[60]	2022	Effect of Concurrent Training on Body Composition and Gut Microbiota in Postmenopausal Women with Overweight or Obesity	France	Randomized controlled trial	17				N/A
[61]	2022	Exploring the Effects of Six Weeks of Resistance Training on the Fecal Microbiome of Older Adult Males: Secondary Analysis of a Peanut Protein Supplemented Randomized Controlled Trial	USA	Secondary analysis of 14 males that completed six weeks of resistance training		14			N/A

significantly reduced ($P = 0.03$) [97], *Clostridium subcluster XIVa* shows a reduction in women who perform endurance exercise for twelve weeks meanwhile the genus *Bacteroides* shows a significantly increased [98].

When comparing the relative abundance of control and exercise groups, as shown in Table 3, authors inform significant differences in *Bacteroides* and *Subdoligranulum* [89] and a significant increase of *Phascolarctobacterium* and *Mitsuokella* in the exercise group [99]. Differential abundance analysis between two intervention groups conducted at the genus level showed *that Haemophilus, Butyricicoccus, Eubacterium hallii, and Ruminiclostridium* were reduced. In contrast, *Coprobacter*, and uncultured bacterium (from *Rhodospirillales* order) increased in the intervention group compared with the control group (all FDR $P < 0.1$) [28].

Similarly, the results described by Magzal et al. [96] in a cross-sectional study including 39 older adults suffering insomnia and classified into the groups high and low PAL shows that *Bifidobacterium*, *Clostridium* sensu stricto *1*, *Catenibacterium*, *Peptococcus*, *Holdemanella*, and *Butyricicoccus* are among the genera present in more active individuals. Less active people had a higher relative abundance of the genera *Barnesiella*, *Blautia*, *Lachnoclostridium*, *Christensenellaceae R-7* group, and *UCG-005* [96].

Few studies report significant presence or abundance at the species level, Fielding, and collaborators showed that *Faecalibacterium prausnitzii*, *Barnesiella intestinihominis*, *Bacteroides caccae*, and *Clostridium citroniae* were higher in older adults with high fitness profile; meanwhile, a reduction in *Eubacterium biforme*, *Desulfovibrio D168*, and *Escherichia coli* was observed when compared to the Low Fitness group in a cross-sectional study where 29 older women and men (70 years) performed a short physical battery [92].

Various studies aimed to establish a correlation between important indicators of physical activity status such as maximal oxygen consumption or VO2 peak (ml/kg/min) [90, 97, 100], based on the results of cardiorespiratory fitness, older adults were divided into two or three functional groups, where those with higher values oscillate between 22.17 ± 0.51 [90], 23.2 ± 5.8 [100], and 27.3 ± 4.6 (ml/kg/min) [97]. Older people with less VO2 peak values are shown as statistically significantly lower. Correlation analysis exhibit that gut dysbiosis is associated with the reduced exercise capacity of elderly patients with hypertension [90].

Similar findings are observed in a randomized controlled trial [100], where 17 aged adults were assigned to exercise (HIT + RT) or a control group, eventually, after 12 weeks program measurement of VO2max ($mL \cdot kg - 1 \cdot min - 1$) was performed, posterior analysis showed that the Shannon's index was positively correlated with VO2max changes; results suggest an association between microbiota richness and cardiorespiratory fitness improvements [100]. Other correlation analyses between the baseline relative abundance of specific microbiota families and the changes in body composition and cardio metabolic parameters showed that *Bifidobacteriaceae* abundance was positively correlated with fat mass and negatively with muscle mass. Equally, *Paraprevotellaceae* and *Prevotellaceae* were negatively correlated with fat mass and positively with muscle mass [100].

Table 3 shows a trend in the method employed to determine gut microbiota composition using the 16S rDNA amplicon based NGS. Only one selected study indicated Next Generation Sequencing (NGS) [70]. Meanwhile, another reported analyzing available data from The American Gut Project (AGP) [94, 101]. Table 3 summarizes the results related to the diversity and composition of the gut microbiota. Only significant differences between groups are described.

A deeper analysis of functional predictions shows that some metagenomic functions were significantly different between exercise and control periods ($P < 0.05$; FDR < 0.3). Based on the KEGG database, functions related to genetic information processing and nucleotide metabolism were overrepresented after a 5-week endurance exercise program in older Japanese men [97]. A similar analysis shows some crucial differences in 26 metagenomic functions when comparing high-fitness (HF) and low-fitness (LF) aged people. The authors emphasize that the expression of glutathione peroxidase (K00432; GPx) was higher, whereas the remaining 25 functions were lower in HF when compared with LF. GPx was the most highly expressed function (2 to 20-fold increased) compared to all other significant KEGG IDs. [92].

The physical activity frequency is also related to the relative abundance of microbial pathways. Zhu and collaborators suggest that regular exercise significantly modulated microbial function in older people because of the functional analysis performed in samples recovered from the American Gut Project [28]. In synthesis, the relative abundances of 18 pathways were significantly higher. In comparison, the abundances of 5 of those pathways were significantly lower in the daily or regular exercise group (DRE) than in the never or rare exercise group (NRE). These pathways include vitamin-related pathways, nucleotide metabolism-related pathways, glucose metabolism, and amino acid metabolism [94]. Some studies have involved direct quantification methods, such as untargeted metabolomics; Results reported by Castro-Mejía et al., describe significantassociations (>|0.2| r) for ten gut metabolites and five plasma metabolites with lifestyle

Table 3 Synthesis of results

Ref	Type of intervention		Type of analysis of the Gut Microbiota	Diversity metrics		Composition					
	Exercise Group	Control Group		Alpha diversity	Beta diversity	Relative abundance (Significant differences)					
						Phylum	Class	Order	Family	Genus	Species
[49]	5-week endurance exercise program—with five weeks endurance control group—three cycle ergometer sessions per week	Physical activity level monitoring	16S rDNA amplicon generation Region V3-V4	None of the changes in α-diversity indices were different between the exercise and control periods	The plots indicated that the gut microbial communities were almost identical between the exercise and nonexercise periods (PERMANOVA, $P > 0.05$	NO	NO	NO	NO	↑ *Oscillospira* during the exercise period in the control first group ($P = 0.003$) ↓*C. difficile* during the exercise periods in both groups ($P = 0.03$) and ($P = 0.01$)	NO
[50]	Weber's classification system [a]: Class A (average exercise capacity), Class B, and Class C (reduced exercise capacity)	Weber classification Class A- B- C	16S rDNA amplicon generation Region V4	No significant differences were detected among the three groups in alpha diversity measures ($p > 0.05$)	Weber A samples were separate from the other groups (ANOSIM pairwise comparisons generated an $R > 0.5$, $p < 0.05$	NO	↑ *Betaproteobacteria* in the Weber A group	↑ *Burkholderiales* and the family *Alcaligenaceae* in the Weber A group	↑ *Ruminococcaceae* in the Weber A group	↑ *Faecalibacterium* in Weber A group ↑ *Escherichia_Shigella* in Weber C group ↑ *Blautia* and *Eubacterium hallii* in Weber B group	↑ *Escherichia coli* in Weber C group
[51]	The objective measure of physical activity with multi-sensor for a typical 7-day period following	N/A	16S rDNA amplicon generation Region V4	No difference in alpha diversity was reported	Step count and self-reported PA were consistently associated with β-diversity as determined by unweighted Unifrac	NO	NO	NO	NO	NO	NO
[59]	Aerobic exercise training (AE) or trunk muscle training (TM)	N/A	Terminal restriction fragment length polymorphism (T-RFLP) analyses	N/A	N/A	NO	NO	NO	NO	↑ *Bacteroides* ↓*Clostridium subcluster XIVa* in the AE group. The relative abundance of *Clostridium cluster IX* was only significantly increased in the TM group	NO

Table 3 (continued)

Ref	Type of intervention		Type of analysis of the Gut Microbiota	Diversity metrics		Composition					
	Exercise Group	Control Group		Alpha diversity	Beta diversity	Relative abundance (Significant differences)					
						Phylum	Class	Order	Family	Genus	Species
[53]	Short physical performance battery (SPPB)—HF / LF	N/A	16S rDNA amplicon generation—Region V4	Measures of alpha diversity were not significantly different when comparing groups	N/A	NO	NO	NO	↑Prevotellaceae and ↑ Paraprevotellaceae in the HF group	↑*Prevotella*, ↑*Barnsiella*, and ↑*Phascolarctobacterium* in the HF group	*Faecalibacterium prausnitzii*, *Barnesiella intestinihominis*, *Bacteroides caccae*, and *Clostridium citroniae were* higher in the HF group -*Eubacterium biforme*, *Desulfovibrio D168*, and *Escherichia coli* were lower in HF
[54]	Two fitness phenotypes, high fitness (HF) and low fitness (LF)	N/A	16S rDNA amplicon generation Region V3	NO	Significant correspondence ($p = .04$) and dissimilarities ($p = .01$) in gut microbiota composition in connection with the two physical phenotypes	NO	NO	NO	NO	NO	NO
[55]	PAL by the FGAS scale: community-dwelling older adults (older adults) and physically active senior orienteers (senior orienteers)	N/A	NGS	No difference in alpha diversity in terms of the Shannon index was observed between the groups	N/A	NO	NO	NO	NO	NO	*Faecalibacterium prausnitzii* and *Bilophila unclassified* were significantly different for 8/15 covariates or combinations of covariates

Table 3 (continued)

Ref	Type of intervention		Type of analysis of the Gut Microbiota	Diversity metrics		Composition					
	Exercise Group	Control Group		Alpha diversity	Beta diversity	Relative abundance (Significant differences)					
						Phylum	Class	Order	Family	Genus	Species
[56]	**Exercise frequency** Daily exercise group, Regular exercise group (DROE), Occasional exercise group, rare exercise group, never exercise group (NROE)	N/A	Data recover from AGP [57]	OTU numbers were 207.2 and 195.2 ($p < .001$), while the Shannon indices were 5.681 and 5.508 ($p < .001$) in the DROE and NROE groups, respectively. Microbial α-diversity was significantly affected by exercise in overweight individuals	N/A	NO	NO	NO	↓ *Actinomycetaceae, Desulfovibrionaceae, S24-7, Pseudomonadaceae, Barnesiellaceae*, and *Oxalobacteraceae* with exercise frequency. Gradually ↑ *Campylobacteraceae, Fusobacteriaceae, Turicibacteraceae, Paraprevotellaceae, Clostridiaceae, Peptostreptococcaceae, Corynebacteriaceae*, and *Bacteroidaceae* with exercise frequency	NO	NO
[54]	Habitual PA behaviors were assessed by wearing a monitor for 24 h per day	N/A	16S rDNA amplicon generation Regio v3-v4	N/A	N/A	NO	NO	NO	NO	NO	NO

Table 3 (continued)

Ref	Type of intervention		Type of analysis of the Gut Microbiota	Diversity metrics		Composition					
	Exercise Group	Control Group		Alpha diversity	Beta diversity	Relative abundance (Significant differences)					
						Phylum	Class	Order	Family	Genus	Species
[48]	lifetime high-endurance athletes (LA) and subjects who meet the minimum recommended physical activity levels (CTRL)	N/A	16S rDNA amplicon generation	No differences in alpha diversity indices between athletes and non-athletes	N/A	NO	↑ Cytophagia ($p=0.03$) in the CTRL group compared to the LA group	NO	A significantly different abundance of *Bacteroidaceae* in the CTRL group compared to the LA group ($p=0.002$) and *Clostridiales Incertae Sedis XI* ($p=0.01$)	Significant differences were observed in the relative abundance of genus *Bacteroides* ($p=0.002$) between the CTRL and LA group *Phascolarctobacterium* ($p=0.01$) *Subdoligranulum* ($p=0.02$)	*Blautia, Faecalibacterium Bacteroides*, and *Roseburia* formed a significantly different part of all bacteria, 9.3% (7.9–10.8) in the CTRL group compared to 6.2% (5.4–7.2) in the LA group ($p=0.001$)
[58]	PAL was assessed objectively over two weeks of daily measurement using an accelerometer Step-count: more active/less active groups	N/A	16S rDNA amplicon generation region V4	NO	Microbiota divergence between individuals (Beta diversity, PERMANOVA, $p<0.394$)	NO	NO	NO	NO	NO	NO
[59]	An 8-week exercise training randomized controlled trial was conducted. This exercise training program consisted of aerobic and resistance exercises	N/A	16S Region V4	NO	N/A	NO	*Clostridia* ↓ compared with the control group after intervention. Significant difference for *Betaproteobacteria* between the two groups ($F=5.149$, $P=0.047$)	*Bifidobacteriales* ↓ of the control group after intervention. *Burkholderiales* showed an increase in the exercise group and a reduction in the control group,	↑*Acidaminococcaceae* in the exercise group, ↑ *Bacteroidaceae* in the control group	↑**EXERCISE GROUP** *Phascolarctobacterium* and *Mitsuokella* ↓**CONTROL GROUP** *Bacteroides* and *Parabacteroides*	

Table 3 (continued)

Ref	Type of intervention		Type of analysis of the Gut Microbiota	Diversity metrics		Composition					
	Exercise Group	Control Group		Alpha diversity	Beta diversity	Relative abundance (Significant differences)					
						Phylum	Class	Order	Family	Genus	Species
[15]	One year of intervention by promotion of physical activity (IG)	Control group (1-year follow-up	16S rDNA amplicon generation Region V2—V4	NO	N/A	NO	NO	NO	NO	**Intervention Group** ↓ *Haemophilus, Butyricicoccus, Eubacterium hallii*, and *Ruminiclostridium 5* ↑*Coprobacter* (FDR $P < 0.1$)	NO
[60]	Training program HIIT + RT: three times per week for 12 wk	CONT	16S rDNA amplicon generation Region V4	NO	PCoA of the unweighted Unifrac distance matrices showed that the pre-and post-intervention microbiota composition changed in most patients from the HIIT + RT group, whereas it remained stable in the CONT group	NO	NO	NO	NO	NO	NO

[a] Based on the peak VO2 values obtained in test

↑: Increase

↓: Decrease

co-variables, such as steps per day which correlated positively with mono and di-saccharides metabolism and negatively with amino acid and lipid metabolism. Also, they did not find an essential difference in the concentrations of Short-Chain Fatty Acids (SCFA) from the fecal metabolome according to the high o lower fitness phenotype [93]. In contrast, Magzal, and collaborators report higher concentrations of total SCFA in people with lower physical activity levels [96]; here, acetate was the most prevalent SCFA in both groups. Analysis of the difference in these volatile compounds revealed that the less active group had significantly higher concentrations of propionate, isobutyrate, and valerate compared with the more active group. The magnitude of the difference in concentration between the study groups was higher for propionate ($\eta 2 = 16$). The less active group also had significantly higher concentrations of total fecal SCFAs, compared to the more active activity group, with a medium effect size ($\eta 2 = 08$) [96].

Finally, we identified a high variability in the frequency of physical activity both in longitudinal and cross-sectional studies. In brief, randomized trials included exercise protocols between a) 5 weeks of endurance exercise program comprising three ergometer sessions per week [97]; b) Supervised resistance training sessions, twice weekly for six weeks [66]; c) 8-week exercise training randomized controlled trial comprised aerobic and resistance exercise [99]; d) 12-week comparative trials, between aerobic exercise training or trunk muscle training [98]; 12-week training program included high-intensity training and resistance training three times per week [100]. We consider this data is not enough to describe the effect of different exercise intensities and durations on the composition and function of the gut microbiota of older people.

Discussion

This review summarizes 15 studies involving physical activity, exercise, and gut microbiota changes. In brief, three randomized control trials and 11 cross-sectional trials were analyzed to determine whether performing an exercise program, or higher levels of PA, are consequent to changes in the diversity, abundance, and functional parameters of the gut microbiota of older adults. Similar to reports from other systematic reviews, there are no significant shifts in diversity metrics (Alpha and Beta). Here, only one study from recovery data reported a significant difference in Alpha diversity from overweight people with higher PAL. Contrary to similar findings reported by Barton et al. [63], the microbiota alpha diversity of elderly athletes defined by the Shannon and Simpson index and the Chao1 index did not differ from that of the controls [89].

The abundance of some bacteria is higher in aged people, after an exercise program, or in comparison with control groups, especially at the genus level (Table 3). Some of these bacteria are from the *Lachnospira* and *Lachnospiraceae NK4A136* group, these microbiota members have been described as potentially beneficial [102], because they are producers of SCFA [28], and the synthesis of these organic acids is usually linked to important roles in maintaining colonic host health as an energy source, regulator of gene expression, and anti-inflammatory agents [103], which might be beneficial for the host.

Similar to other studies in non-older adults, some results included in this systematic review suggest that regular exercise significantly modulated microbial function in elderly individuals the data proportionate so far is limited and few studies have included extra analysis such as metabolomic assays or metagenomic approach, where microbial compounds and relative pathways related to physical activity could discover [94], in contrast, other studies including non-older adults have reported significant findings by using specific analysis techniques and combination of omics technologies [69, 104, 105].

Here we highlight the association between the relative abundance of gut microbiota and physical function [99] and a reduced exercise capacity that is negatively associated with the core gut microbiota [90, 91]. We also identified in this systematic review that similar to results presented in cross-sectional studies with young adults [58, 106], consumption of oxygen by older men and women is correlated with species richness and higher diversity of bacterial members of the gut microbiota [90, 97, 100], which reinforce the hypothesis that effect of PAL is more related to functional outcomes rather than compositional indicators (such as diversity or abundance) further investigation is required.

Associations between physical activity and gut microbiota have yet to be extensively studied in older adults. Existing publications focusing on young adults and athletes show consistent results related to the production of SCFAs [63]. Also, bacteria such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzi* have been described in the past [107]. In addition, the health status of older adults involved in biological and gut microbiota studies might be a limiting factor since including people with insomnia [96], Arterial Hypertension, Dyslipidemia, Hyperglycemia, Prostatic Hyperplasia [97], Primary Hypertension [90], Osteoporotic Fractures [91], Overweight and Obesity [94], Cardio Vascular Disease, Type 2 Diabetes mellitus [95] is present in this systematic review. These medical conditions have been reported as modulators of gut microbiota composition [23, 108, 109]. However, data availability for older adults is limited, and

comparing healthy and unhealthy subjects could be complicated to perform.

Although the response to exercise and augment of PAL has been extensively studied in diverse biologic systems, such as the mitochondria, the muscle, the liver, and the neurologic system, among others, it is still unclear whether these changes are related to the gut microbiota in older adults. Past reviews and animal studies have linked the possible physiological response to exercise with the community of microorganisms that inhabit the gut [53, 110, 111]. We did not find consistent results that may reflect the modifications of the gut microbiome in other physiological systems. Some bacteria taxa whose abundance changed are beneficial for aged people (Table 3), such is the case of the genus *Oscillospira* which is a promising candidate for the next generation -of probiotics because of its capacity to produce butyrate [112]. *Faecalibacterium* and *Coprococcus* have been correlated with host quality of life indicators in humans diagnosed with depression [113], and some species of the genus *Bacteroides* and *Parabacteroides* are more extraordinary producers of γ-aminobutyric acid (GABA) [114]. Similar findings are described for *Faecalibacterium prausnitzi* that, besides promoting the production of metabolites, have been related to the decrease of inflammatory markers in patients with Alzheimer's-type dementia [115]. *Eubacterium hallii* is also considered a SCFAs producer, especially propionate [116], thanks to metagenomics. It has been discovered that *Subdoligranulum MGS* (metagenomics species) was co-abundantly found with *Akkermansia muciniphila* [117], a promising biomarker for nutritional status [118].

Otherwise, the study provided by Fielding and collaborators [92] looks to describe a correlation between muscle function and gut microbiota through the colonization of mice with microbiota from highly functional older adults. Although results are inconclusive, authors stated that bacteria taxa at the family-level *Prevotellaceae*, genus level *Barnesiella* and *Prevotella*, and species-level *Barnesiella intestine hominis* might be involved in mechanisms related to the maintenance of muscle strength in older adults [92].

Deeper analysis performed in the studies included identifying metagenomic functions and metabolic pathways to describe some metabolic signatures related to vitamin, amino acid, and glucose functions. In contrast with other reports [33, 119, 120], we did not find an essential association between SCFA and physical activity in older adults. However, very few studies include metabolomic assays, and the data is limited (Fig. 2). This allows

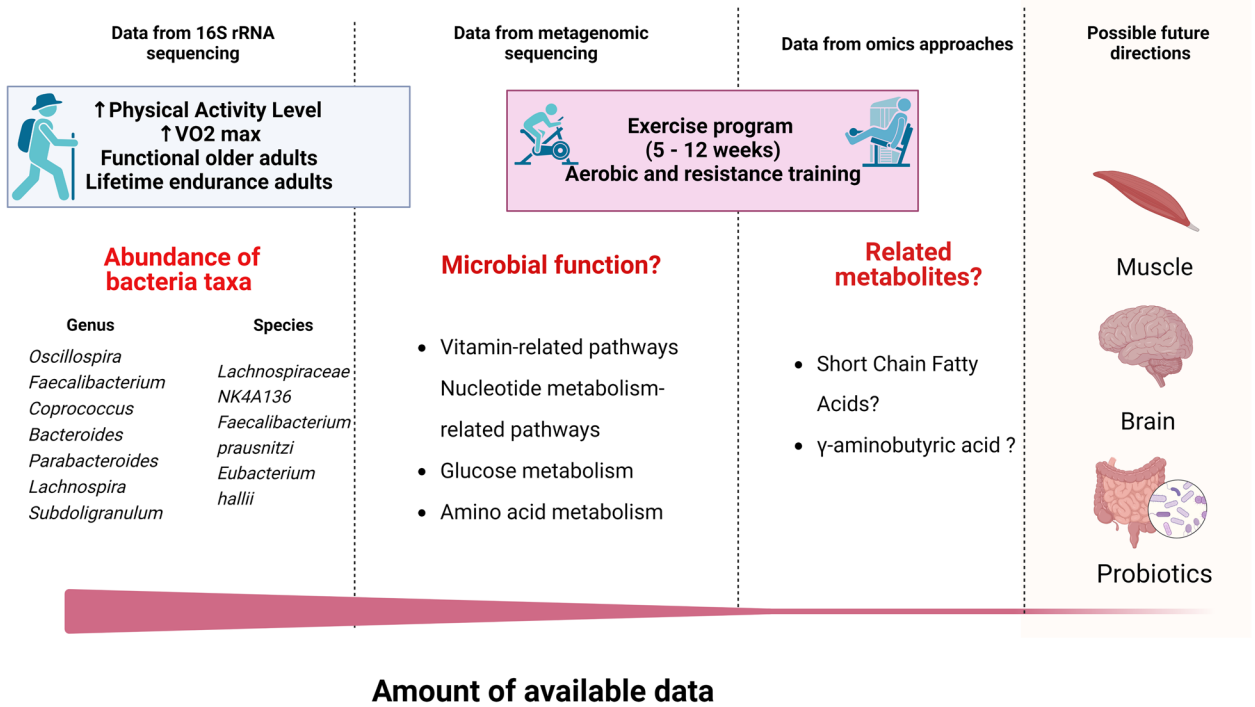

Fig. 2 Schematic representation of the data available from studies included in this systematic review. The amount of information available is mostly related to data from 16S rRNA sequencing and the identification of some bacteria associated to beneficial functions for the host; although very few studies used metagenomic approaches, some bacterial functions could be identified in future studies. Here, we identify only two studies describing SCFAs and results are inconclusive. Future directions could link the already known effect of exercise on brain and muscle function in older adults and the gut microbiome

us to identify that including diverse sequencing methodologies and the analysis of related metabolites such as SCFAs and GABA in combination with metagenomic approximations could help to describe the role of physical activity on the gut microbiota of older adults in future studies.

Information on taxa and functions related to the benefits of performing PA has been relevant in the identification and isolation of probiotic candidates [121]. Also, including omics techniques, would give insights into the mechanisms underlying the effect of exercise on the gut microbiome of older adults and whether it differs from young people.

Conclusions

This review aimed to determine if there is any effect on the gut microbiota of adults older than 65 who start an exercise intervention or improve physical activity level. The studies identified do not address this systematic review's objectives. However, almost all the studies analyzed the diversity and abundance of the gut microbiota; there needs to be more information related to function and metabolic pathways that can be crucial to understand the effect of exercise and physical activity in older adults. It is essential to highlight the lack of randomized controlled trials in this field. Most of the studies included are observational, and interventions were mainly voluntary, based on physical exercise (aerobic or muscular) or to increase physical activity through lifestyle changes (increasing the number of steps). The lack of data related to gut microbiota analysis is a weakness that needs to be addressed in future studies.

Limitations of this review

Authors consider that some limitations of this review included publication bias because of one of the main criteria to report findings related to physical activity and gut microbiota of older adults, which was the statistical significance even though studies with results that do not show statistical significance may be clinically significant, and thus important to the findings of a systematic review. We also consider that the selection and inclusion of cross-sectional studies could be a potential limitation in this review. This is the first time that physical activity, microbiota, and older adults are compared in a systematic review.

Abbreviations

OTUs	Operational Taxonomic Units
WHO	World Health Organization
ASV	Amplicon Sequence Variance
T2D	Type 2 Diabetes
SVs	Sequence Variants
PRISMA	Preferred Reporting Items for Systematic Reviews
AGP	American Gut Project
FGAS	Frändin–Grimby Activity Scale
PAL	Physical Activity Level
NGS	Next-generation sequencing
PA	Physical Activity
HF	High Fitness
LF	Low fitness
HIIT	Hight Interval Intensity Training
RT	Resistance Training
SCFA	Short-Chain Fatty Acids
GABA	γ-Aminobutyric acid
KEGG	Kyoto Encyclopedia of Genes and Genomes
DRE	Regular Exercise Group
NRE	Never or Rare Exercise group

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12877-023-04066-y.

Additional file 1: Supplementary Table 1. ROBINS-I risk of bias assessment summary: review authors' judgements about each methodological quality item for each non-randomized included study in this review.

Additional file 2: Supplementary Table 2. A revised tool to assess risk of bias in randomized trials (RoB 2) summary: review authors' judgements about each methodological quality item for each randomized included study in this review.

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Authors' contributions

VA and JDR: designed the study, conducted the analysis and drafted the manuscript. PJ and EM: extracted the information and validated the results. All authors approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Publisher's Note

CAPÍTULO 2

El microbioma intestinal ha ganado atención en los últimos años debido a su impacto en la salud y el rendimiento físico de los deportistas. Este capítulo se centra en el análisis comparativo del microbioma intestinal de tres grupos: levantadores de pesas, ciclistas de ruta y sujetos no deportistas, con el fin de identificar patrones únicos que puedan estar asociados con diferentes disciplinas deportivas y estilos de vida.

La variabilidad en la composición del microbioma intestinal puede influir en el rendimiento deportivo y la salud general. La identificación de firmas microbianas específicas en deportistas de diferentes disciplinas puede proporcionar información valiosa sobre cómo el ejercicio físico afecta el microbioma y, en consecuencia, el bienestar del hospedador.

Para cumplir con el objetivo específico dos de esta tesis, se diseñó un estudio transversal que incluyó 16 muestras de levantadores de pesas, 13 de ciclistas de ruta y 15 de sujetos no deportistas. Se establecieron criterios de inclusión (adultos jóvenes de 18 a 35 años, entre otros) y exclusión (antecedentes de enfermedades metabólicas o cardiovasculares, entre otros). Se recopiló información sociodemográfica, antecedentes médicos, hábitos de vida y frecuencia de entrenamiento para deportistas, así como el nivel de actividad física mediante el IPAQ para los no deportistas. Las muestras biológicas recolectadas incluyeron sangre total y heces fecales. Para la toma de muestras sanguíneas, se siguió un protocolo de ayuno previo y recolección por personal capacitado. Las muestras fecales se recolectaron en el hogar de los participantes y/o en lugares de concentración de deportistas, utilizando un kit específico para evitar contaminación.

Los procedimientos metagenómicos comenzaron con la extracción de DNA mediante técnicas estandarizadas. Posteriormente, se prepararon las librerías para ser secuenciadas en la plataforma Illumina HiSeq 2500, y los datos obtenidos se analizaron mediante herramientas bioinformáticas como FastQC, Trimmomatic, Bowtie2 y Kraken2.

Los resultados detallados del análisis metagenómico no solo revelaron diferencias significativas en la composición bacteriana del microbioma intestinal entre los grupos de ciclistas, levantadores de pesas y no atletas, sino también en las firmas virales. En los levantadores de pesas se observó un aumento significativo en la abundancia de virus de la familia *Siphoviridae*, mientras que en los ciclistas se destacó la presencia de *Mimiviridae*. En contraste, los no atletas mostraron una menor diversidad de familias virales, con una predominancia de *Mimiviridae* en correlación exclusiva con *Mollicutes*.

Además, un hallazgo relevante fue la prevalencia incrementada de virus *crAss*-like en individuos no atletas, en comparación con ciclistas y levantadores de pesas. Estos *crAss*-like virus, pertenecientes al orden *Crassvirales*, están presentes en abundancia en la microbiota intestinal humana, aunque su papel aún no se comprende completamente, lo que sugiere la necesidad de investigaciones futuras para confirmar y ampliar estos resultados.

El análisis de correlación reveló que las interacciones entre bacterias y virus en estos grupos se caracterizan principalmente por correlaciones positivas, lo que indica interacciones complejas

entre estos microorganismos. En los ciclistas, se observó una distribución predominante de fagos de *Faecalibacterium* y *Riboviria*, mientras que los no atletas y levantadores de pesas mostraron una distribución viral similar, incluyendo la presencia de virus crAss-like y *crAssphage* no cultivados.

Aunque la abundancia total de virus fue baja, representando aproximadamente el 0.12% del microbioma intestinal, estos resultados sugieren un papel modulador de la actividad física sobre el viroma intestinal, destacando la necesidad de estudios adicionales que profundicen en la función de los crAss-like virus en poblaciones de deportistas. Los resultados a mayor detalle pueden ser encontrados en el siguiente artículo:

- **Artículo 3:** Aya V, Vega LC, Muñoz E, Muñoz M, López DF, Guzmán MP, et al. Divergent Gut Microbioma: Archaeal and Bacterial Signatures Unveil Unique Patterns in Colombian Cyclists Compared to Weightlifters and Non-Athletes. Adv Biol. n/a(n/a):2400069.

CAPÍTULO 2: Caracterización y comparación taxonómica del microbioma intestinal de deportistas y no deportistas colombianos

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Divergent Gut Microbiota: Archaeal and Bacterial Signatures Unveil Unique Patterns in Colombian Cyclists Compared to Weightlifters and Non-Athletes

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Engagement in physical activity, across various sports, promotes a diverse microbiota in active individuals. This study examines the gut microbiota of Colombian athletes, specifically weightlifters ($n = 16$) and road cyclists ($n = 13$), compared to non-athletes ($n = 15$). Using Kruskal–Wallis tests, the physical activity level of a group of non-athletic individuals and the sports experience of a group of professional athletes is analyzed. The median age of participants is 24 years, comprising 25 men and 19 women. The microbiota is collected using fecal samples. Participants provided these samples during their pre-competitive stage, specifically during the concentration phase occurring two weeks prior to national competitions. This timing is chosen to capture the microbial composition during a period of heightened physical preparation. Questionnaire responses and microbial composition assessments identify disparities among groups. Microbial composition analysis explores core microbiome, abundance, and taxonomy using Pavian, MicrobiomeAnalyst 2.0, and GraPhlAn. ANCOM-BC2 reveals differentially abundant species. Road cyclists exhibit decreased Bacteria and increased Archaea abundance. Phylum-level variations included Planctomycetes, Acidobacteria, and Proteobacteria, while Bacteroidetes prevailed. Key families influencing gut microbiota are Bacteroidaceae, Muribaculaceae, and Selenomonadaceae. Weightlifters exhibit unique viral and archaeal community connections, while cyclists showed specialized microbial interplay influenced by endurance exercise. Correlation network analysis emphasizes distinctive microbial interactions within athlete groups, shedding light on the impact of physical activities on gut microbiota and athlete health.

1. Introduction

The gut microbiome's composition is influenced by genetic factors, diet, age, environment, body composition, sedentary lifestyle, and physical activity, sports, athletic performance, among others.[1–3] Physical activity (PA), including exercise and daily movements, has acquired attention for its potential to modulate the gut microbiome, showing a more diverse microbiota in physically active individuals compared to sedentary ones.[2,4] Different sports, involving endurance exercise, strength training, or flexibility exercises, contribute to this effect.[5] Physically active individuals, both women, and men, exhibit an increased presence of beneficial bacteria such as *Lactobacillus*, *Coprococcus*, *Faecalibacterium prausnitzii*, *Bifidobacterium adolescentis group*, *Bifidobacterium longum group*, *Lactobacillus sakei group*, and *Blautia*.[4,6–8] These microbiota members are well known for being markers of good intestinal health and have been identified as beneficial bacteria in human health and sports performance.[9]

According to a recent study, sedentary behavior is linked to a decrease in gut microbiota richness, implying

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that exercise patterns, like taking breaks from sitting, can influence gut microbiota composition.[10] Sedentary individuals show lower diversity in their gut bacterial and fungal microbiota compared to those with an active lifestyle. Studies on Chinese and Spanish sedentary populations reveal specific microbial differences,[10–12] including higher Firmicutes and Ascomycota levels, and lower Actinobacteria, Proteobacteria, and Basidiomycota levels. Sedentary individuals exhibit reduced diversity and network complexity in their gut microbiota compared to active individuals.[13] While mutualism and co-occurrence interactions are similar, those with an active lifestyle display greater competitiveness. A meta-analysis on non-athletes shows that *Bacteroides*, particularly *Bacteroides uniformis*, is prevalent, but individuals with low physical activity levels (PAL) have elevated *Alistipes putredinis* abundance compared to their active counterparts.[14]

With different studies aimed at examining the composition of microorganisms from disciplines like cycling,[15] running,[16,17] swimming,[18] rugby,[19] among others[20] the gut microbiota of athletes has been studied in the past.[21] A recent screening of 185 shotgun metagenomic data recovered from athletes engaging in high-intensity efforts (efforts comprised between >6 s and 1 min, with preponderance of the "glycolytic pathway"), and mainly, endurance intensive efforts (for exercise bouts longer than 1 min, with preponderance of the "oxidative phosphorylation pathway")[19,22–25] revealed a high prevalence of *Faecalibacterium unknown_species, Bacteroides uniformis, Faecalibacterium prausnitzii, Bacteroides unknown_species, Blautia unknown_species, Eubacterium rectale, Phocaeicola vulgatus*, and *Eubacterium hallii*.[26] Through the identification of precise genes and metabolic pathways within the microbial community,[25] some of these bacterial species are commonly recognized as producers of short-chain fatty acids (SCFA) like butyrate, which serves as a primary energy source for enterocytes through the fermentation of glucose.[25,27]

While some studies have explored the connection between physical activity and the gut microbiome, limited information exists on strength sports.[23] Strength and endurance sports show distinct physiological adaptations based on exercise type. Endurance exercise places strain on the cardiorespiratory system, sustaining for a prolonged duration. Physiological adaptations include increased stroke volume, cardiac output, oxygen uptake, hemoglobin levels, and enhanced muscle capillary density.[28,29] Previous research has suggested physiological links between the muscular system in endurance activities and gut microbiota.[23,30,31]

Strength training exercises, such as weightlifting or resistance training, focus on the musculoskeletal system and require high intensity and shorter durations.[29,32] Few strength athletes have been involved in microbiome studies, an observational study from Korea described the differential abundance across bodybuilders and non-athletes, with significant changes in the relative abundance of *Faecalibacterium*, *Bifidobacterium*, *Parasutterella*, *Lactobacillus sakei*, *Blautia wexlerae*, and *Eubacterium hallii*.[33]

Current research on gut microbiota composition predominantly focuses on endurance sports like cycling or running, often with participants from the non-South American region, thereby creating a significant knowledge gap. To address this, it is crucial to incorporate data from diverse geographical locations and various sports disciplines, including strength-based sports with a proper comparison with non-athletes. This broader approach would enhance our understanding of the relationship between the gut microbiome and athletic activities.

In this context, we conducted a cross-sectional study to characterize the microbial community of strength (weightlifters) and endurance athletes (cyclists), alongside a group of non-athletic subjects. Our study aimed to examine the gut microbiota composition and differences among three distinct groups using shotgun metagenomics.

2. Results

2.1. Physical Activity Level and Sport Variables

This study involved three distinct participant groups: professional weightlifters (n = 16), road cyclists (n = 13), and non-athlete subjects (n = 15), comprising 25 men and 19 women. Subsequent statistical analyses were conducted on the collected data to explore potential disparities among non-athletes, cyclists, and weightlifters.

The international questionnaire on physical activity level (IPAQ) for non-athletes and sports experience for athletes, along with the demographic questionnaire, received complete responses from all 44 participants. All athletes were actively involved in Colombian leagues, providing training reports with professional experience with a median of 8.0 years ± 2.50 for weightlifters and 5.0 ± 1.57 for cyclists (**Table 1**). Additionally, they actively participated in a preparatory cycle for either national or international competition, remaining in the same vicinity throughout the study (Figure S1, Supporting Information). Cyclists reported a greater daily training duration (median of 4.0 ± 0.95 h day^{-1} vs 2.0 ± 1.29 h day^{-1}) while maintaining a similar training frequency of 6 days per week in both groups. Weightlifters engaged in training sessions that included power and strength exercises performed at an intensity close to 85% of their maximum strength.

In gender-based comparisons, only height exhibited statistically significant disparities (p-value: 0.0009). Results indicated no notable gender differences in body mass index (BMI), weight, or other study variables. The PAL of non-athletic subjects, measured by IPAQ, is presented in Table S1 (Supporting Information). The results indicate a low-to-moderate physical activity level, accompanied by a significant amount of sedentary behavior (≥450 min per weekday). According to the findings in Table 1, weightlifters and non-athletes had a similar median age and BMI, whereas road cyclists exhibited a significantly lower age and BMI (p-value: 0.00045).

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Table 1. Descriptive data from Colombian athletes and non-athletes.

	Non-athletes $n = 15$		Weightlifters $n = 16$		Cyclists $n = 13$	
	Median	IQR	Median	IQR	Median	IQR
Age (years)	25.0	8.0	27	6.0	20.0	1.5
Body weight (Kg)	62.4	16.1	64.0	17.5	61.5	8.9
Height (cm)*	160.3	10.0	164	11.0	169.3	10.65
BMI (Kg m^{-2})*	24.0	3.8	24.9	3.57	20.6	1.43
Training frequency (h day^{-1})*	–	–	2.0	2.0	4.0	0.75
Training load (days $week^{-1}$)	–	–	6.0	0.0	6.0	0.5
Years as a professional athlete*	–	–	8.0	5.0	5.0	2.5

IQR = Interquartile range; h day^{-1} = hours per day; BMI = Body mass index; *p-value: ≤ 0.05 = Statistical differences between non-athletes, weightlifters, and cyclists.

2.2. Microbial Composition of Colombian Athletes and Non-Athletes

After acquiring data through the R package Pavian,[34] our subsequent step involved extracting clade reads from each taxonomic rank. Following this, we conducted calculations to determine the relative abundance, representing the ratio of a specific microbial taxon to the entire microbial community in a sample.

An in-depth analysis of the microbial composition revealed distinct patterns in the relative abundances of Bacteria and Archaea at different taxonomic levels. At the domain level, Bacteria predominated, constituting 96.4% of the overall microbiota, while Archaea accounted for 1.5%, and Viruses were present at 0.12%. Further exploration at the phylum level uncovered noteworthy differences among the studied groups. The most abundant bacterial phyla included Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Euryarchaeota, while Archaea were primarily represented by Euryarchaeota, Crenarchaeota, Thaumarchaeota, and *Candidatus Korarchaeota*. Of particular interest was the observation that road cyclists exhibited a substantial decrease in bacteria abundance coupled with an increase in Archaea, a pattern not as pronounced in weightlifters and non-athletes.

Significant variation emerged at the phylum level across the three groups (p-value: ≤ 0.05) primarily in the less abundant taxa (**Figure** 1A): Planctomycetes, Acidobacteria, Chloroflexi, Cyanobacteria, Kiritimatiellaeota, Armatimonadetes, Candidatus_Saccharibacteria, Proteobacteria, Thaumarchaeota, and Elusimicrobia.

Conversely, Bacteroidetes exhibited the highest relative abundance (Median: 59.91%, IQR: 21.16) across the samples, followed by Firmicutes (Median: 28.29%, IQR: 12.45), Proteobacteria (Median: 5.57%, IQR: 3.55), and Actinobacteria (Median: 2.38%, IQR: 2.29). Euryarchaeota (0.26% ± 0.54) ranked among the top ten most abundant phyla across all individuals. Furthermore, MicrobiomeAnalyst 2.0 was employed for core microbiome analysis at the family level from all 44 samples.[35] Fisher's exact test was used for calculation, comparing the presence or absence of each microbial taxon between samples. The detection threshold, expressed as relative abundance, reached 0.01% with a sample prevalence of 20%. The core microbiome from the entire sample included Ruminococcaceae, Lachnospiraceae, Rikenellaceae, Bacteroidaceae, Prevotellaceae, and Odoribacteraceae as the most abundant microbial taxa (Figure 1B). This sheds light on the microbial characteristics influencing the composition and structure of the gut microbiota in Colombian athletes.

2.3. Comparative Analysis of Gut Microbiota in Cyclists, Weightlifters, and Non-Athletes

The application of MicrobiomeAnalyst 2.0 streamlined the execution of univariate analysis at the family level, involving the extraction of clade reads, data filtering, and statistical analysis using Kruskall–Wallis tests. Detailed results are presented in **Table** 2 and Figures S2 and S3 (Supporting Information). Briefly, the

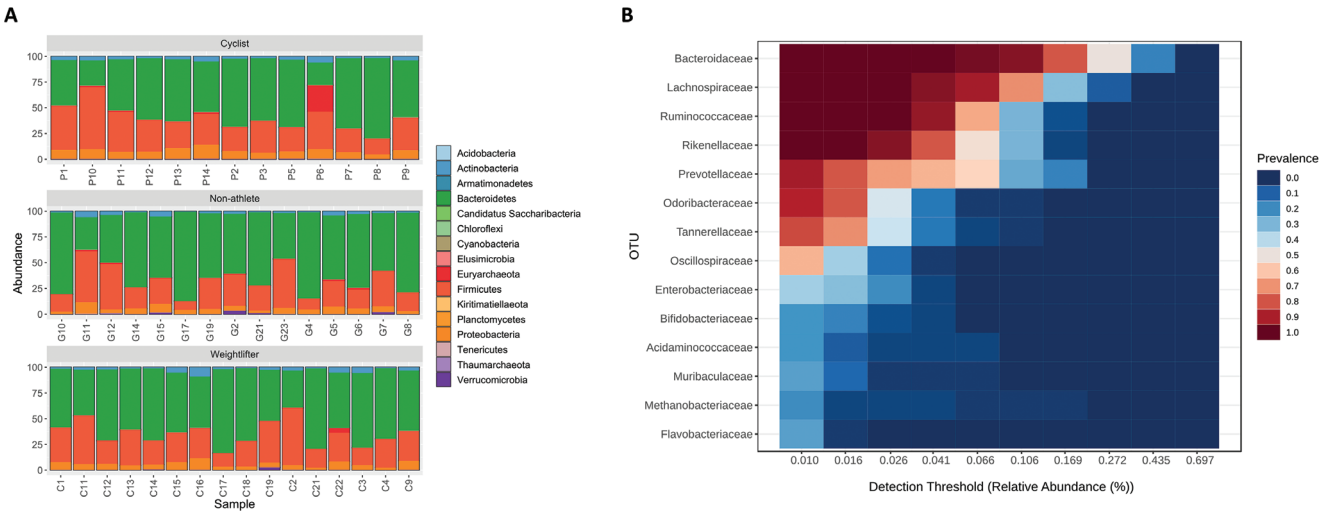

Figure 1. Microbial composition analysis in Colombian athletes: A) Phylum-level abundance comparison: The relative abundance of specific microbial taxa compared to the overall microbial community revealed no discernible variation initially between athletes and non-athletes or between cyclists and weightlifters. B) Core microbiome analysis at the family level: The core microbiome revealed prevalent bacterial families across the whole sample.

Table 2. Univariate analysis results at the family level.

Domain	Family	Weightlifters		Cyclists		Non-athletes		*p*-value	FDR	*Post-hoc*
		M	IQR	M	IQR	M	IQR			
Bacteria	Acidaminococcaceae	0.08	0.08	0.13	0.081	0.071	0.056	NS	NS	N/A
	Bacteroidaceae*	43.9	22.68	23.4	12.63	43.5	22.01	0.0041	0.011	0.022
	Bifidobacteriaceae	0.71	1.58	0.64	0.249	0.67	0.694	NS	NS	N/A
	Clostridiaceae	0.88	0.53	1.09	0.746	0.705	0.751	NS	NS	N/A
	Coriobacteriaceae	0.04	0.04	0.05	0.030	0.05	0.037	NS	NS	N/A
	Enterobacteriaceae	0.97	1.86	0.87	1.111	1.50	2.006	NS	NS	N/A
	Lachnospiraceae	7.44	10.70	8.00	2.683	6.82	5.871	NS	NS	N/A
	Lactobacillaceae	0.29	0.14	0.40	0.257	0.22	0.196	NS	NS	N/A
	Muribaculaceae*	0.0001	0.0001	0.000	0.000	0.000	0.000	0.0004	0.00476	0.163
	Odoribacteraceae	2.16	1.32	1.99	2.239	2.40	1.686	NS	NS	N/A
	Oscillospiraceae	0.29	0.31	0.43	0.463	0.35	0.389	NS	NS	N/A
	Peptostreptococcaceae	0.22	0.11	0.28	0.119	0.24	0.142	NS	NS	N/A
	Prevotellaceae*	5.86	8.93	10.5	13.42	0.66	1.546	0.002488	0.00924	1.0754
	Rikenellaceae	2.17	1.77	1.09	2.735	4.04	4.623	NS	NS	N/A
	Ruminococcaceae	1.41	2.29	1.16	0.895	1.04	1.458	NS	NS	N/A
	Selenomonadaceae*	0.15	0.08	0.22	0.093	0.13	0.088	0.02194	0.03784	N/A
	Spirochaetaceae*	0.18	0.10	0.26	0.059	0.18	0.101	0.00021	0.00464	1.7594
	Veillonellaceae	0.15	0.27	0.25	0.461	0.094	0.195	NS	NS	N/A
Virus	Mimiviridae*	0.005	0.0039	0.01	0.003	0.00	0.005	0.0157	0.02955	N/A
	Siphoviridae*	0.025	0.0143	0.04	0.021	0.02	0.019	0.0178	0.03184	N/A
Archaea	Archaeoglobaceae*	0.012	0.0043	0.02	0.007	0.01	0.005	0.02487	0.04211	N/A
	Halobacteriaceae*	0.030	0.0177	0.04	0.032	0.03	0.022	0.010293	0.02164	N/A
	Haloferacaceae*	0.016	0.0106	0.03	0.021	0.02	0.012	0.00025	0.00464	0.592
	Methanobacteriaceae	0.149	0.4358	0.20	1.032	0.22	1.155	NS	NS	N/A
	Methanocaldococcaceae	0.015	0.0085	0.02	0.009	0.01	0.013	NS	NS	N/A
	Methanococcaceae*	0.022	0.0115	0.03	0.012	0.01	0.013	0.01451	0.02764	N/A
	Methanomassiliicoccaceae	0.007	0.0042	0.01	0.005	0.008	0.004	NS	NS	N/A
	Methanomicrobiaceae*	0.013	0.0085	0.02	0.013	0.012	0.012	0.00207	0.00867	1.888
	Methanoregulaceae*	0.01151	0.0048155	0.015	0.007	0.011	0.003	0.00815	0.01838	N/A
	Methanosarcinaceae*	0.077	0.0186	0.11	0.053	0.06	0.032	0.00173	0.00785	N/A
	Natrialbaceae*	0.026	0.0214	0.03	0.025	0.03	0.017	0.00026	0.00464	N/A
	Nitrosopumilaceae	0.004	0.0033	0.00	0.005	0.00	0.002	0.06553	0.09111	N/A
	Sulfolobaceae*	0.014	0.0067	0.02	0.010	0.01	0.008	0.00241	0.00924	N/A
	Thermococcaceae*	0.040	0.0165	0.07	0.014	0.03	0.020	0.00132	0.00716	N/A
	Thermoproteaceae*	0.005	0.0029	0.01	0.004	0.00	0.003	0.01210	0.02410	N/A

*p-value: ≤ 0.05 = Statistical differences between non-athletes, weightlifters, and cyclists, estimated with the Kruskall–Wallis test. M: Median; IQR: Interquartile range; FDR: False Discovered Rate. NS: Nonsignificant; Post-hoc: After conducting univariate analysis, post hoc comparisons were performed with Bonferroni correction to account for multiple comparisons; N/A: Not Applicable.

relative abundance at the specified taxonomic ranks was calculated and graphed separately for each domain: bacteria, archaea, and viruses (**Figure 2**).

Figure 2A illustrates the relative abundance at the bacterial family level, revealing statistically significant differences among the three groups—weightlifters, cyclists, and non-athletes. Notably, Bacteroidaceae exhibited a relative abundance of 23.4% (IQR: 12.63) for cyclists and ≥40% for weightlifters (43.9%; IQR: 22.68) and non-athletes (43.5%; IQR: 22.0), single factor analysis indicated significant variation between groups (p-value: 0.004; FDR: 0.01). Muribaculaceae (p-value:0.0004; FDR: 0.0047), Prevotellaceae (p-value:0.002; FDR: 0.009), Selenomonadaceae (p-value:0.02; FDR: 0.03), and Spirochaetaceae (p-value:0.00021; FDR: 0.004) bacterial families showed statistical significance, with both a p-value and false discovery rate (FDR) ≤ 0.05, indicating a higher relative abundance in the cyclists' and weightlifters' group. After Bonferroni correction, only the Bacteroidaceae family remains significantly different with a p-value less than 0.05 (Table 2; Figure 2A; Figure S2, Supporting Information). In contrast, Lachnospiraceae, Bifidobacteriaceae, and Lactobacillaceae

Figure 2. Comparative analysis of gut microbiota in cyclists, weightlifters, and non-athletes. A) Bacterial family-level abundance: Relative abundance at the bacterial family level, revealing significant differences in the Bacteroidaceae family. Groups of athletes displayed a higher relative abundance of Prevotellaceae. B) Relative abundance of Archaea families: Bar plots represent the relative abundance of Archaea families across athletes and non-athletes. Non-statistical difference was found. C) Relative abundance of Viral families: The abundance of Myoviridae exhibited similarity (≥60.0%) across groups. D) Distribution and transition of top bacterial species in athlete and non-athlete groups: Sankey plot depicts the distribution and transition of the top 20 abundant bacterial species with higher relative abundance. E) Distribution of top Archeal species: emphasizing the significant prevalence of *Methanobrevibacter smithii*, *M. smithii ATCC 35061*, and *Methanosphaera sp*. F) Distribution of top viral species: Sankey plot graph shows the dominant distribution of *Faecalibacterium phages* and *Riboviria* in cyclists, with a similar viral distribution observed between non-athletes and weightlifters, involving *crAss-like* viruses and uncultured *crAssphage*.

families exhibited comparable relative abundances across all groups. Although the gut microbiota of cyclists displayed a reduced relative abundance of the Rikenellaceae family, this discrepancy did not reach statistical significance.

Sankey plots (Figure 2D–F) illustrate the distribution of the top dominant species. Figure 2D specifically represents the top 20 bacterial species with higher relative abundance, showcasing a significant abundance of *Bacteroidales incertae sedis* for all three groups, and dominant abundance of *Bacteroides* spp., including *Bacteroides fragilis, Bacteroides uniformis, Faecalibacterium prausnitzi*, and *Phocaeicola vulgatus*, with varying information flow between groups, predominantly major abundance between cyclists and weightlifters.

Figure 2B,C display the relative abundance of Archaea and Viral families. Distinct differences in clade reads of various Archaea families among male cyclists were observed (Table 2; Figure S3, Supporting Information). The relative abundance of Methanococcaceae, Methanomicrobiaceae, Methanoregulaceae, and Methanosarcinaceae exhibited higher levels in the group of cyclists compared to weightlifters and non-athletes (*p*-value: ≤ 0.05). Weightlifters (Siphoviridae) and cyclists (Mimiviridae) displayed a significant increase in the abundance of two viral families.

Finally, Figure 2E showcases the dominant Archaea species across gut microbiota samples, emphasizing the abundance of *Methanobrevibacter smithii, M. smithii ATCC 35061*, and *Methanosphaera spp* for all three groups.

2.4. Taxonomic Structure of the Microbiota from Weightlifters, Road Cyclists, and Non-Athletes

Cladograms were constructed using GraPhlAn software,[36] utilizing Kraken reports to conduct a comprehensive analysis of the dominant structure within the gut microbiota of weightlifters, road cyclists, and non-athletes. **Figure 3** provides a visual representation of the circular taxonomy for each group under investigation, revealing distinct characteristics in microbial dominance patterns.

In the cladogram for weightlifters (Figure 3A), there is a predominant presence of genera such as *Bifidobacterium*, *Bacteroides*, and *Prevotella*. Similarly, the cladogram for road cyclists (Figure 3B) showcases an abundance of these taxa, with additional prevalence observed for members such as *Alistipes, Clostridium, Oscillibacter, Ruminococcus*, along with clades from Euryarchaeota and Spirochaetes. Conversely, the cladogram for

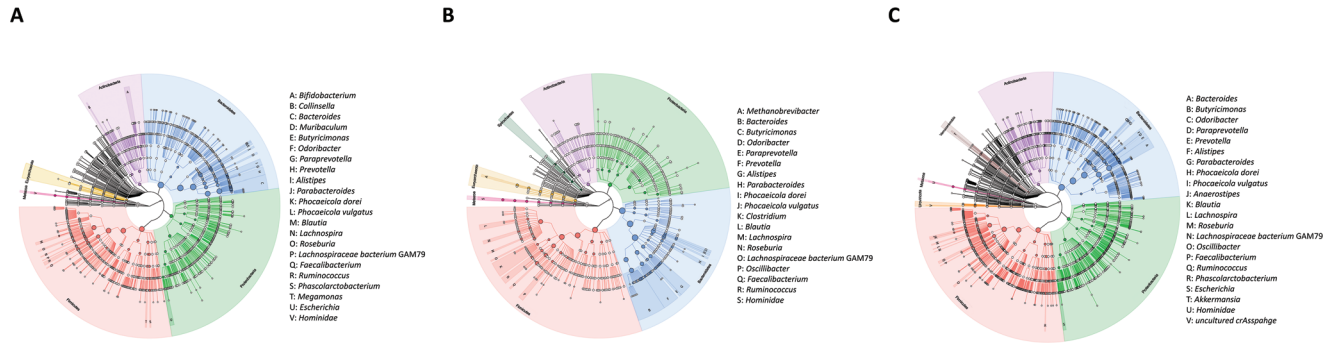

Figure 3. Dominant members of the gut microbiota from the three different groups. This figure represents the dominant taxa for each group included in this study: A) weightlifters, B) cyclists, C) non-athletes. Similarities were observed between weightlifters and non-athletes with a dominant presence of *Akkermansia* (genus) in non-athletic individuals. The presence of Euryarchaeota and Spirochaetes phylum and Methanobrevibacter genus was only identified in cyclists.

non-athletes (Figure 3C) reveals a less abundant presence of these taxa, with a notable increase in *Akkermansia* (genus).

Significantly, the dominant taxa in weightlifters and non-athletes exhibit similarities, indicating comparable microbial profiles between these groups. Conversely, road cyclists demonstrate a distinct taxonomic structure with variations in taxa abundance, emphasizing the potential influence of specific physical activities on gut microbial composition.

3. Differences Across Gut Microbiota are More Related to the Type of Sport

Alpha and beta diversity analyses were carried out to assess variations in the microbial community structure among cyclists, weightlifters, and non-athletes and to determine if significant differences exist. This analysis relied on species reads and taxonomical assignment. Initially, no apparent differences were observed between athletes and non-athletes. However, the Shannon index revealed significant differences in species diversity between cyclists and non-athletes, as well as between weightlifters and cyclists (p-value: $\leq$ 0.001). No discernible distinctions were observed for the ACE and Simpson indexes in **Figure** 4A.

Similarly, beta diversity analysis, assessed through PERMANOVA (Permutational Multivariate Analysis of Variance), indicated a statistically significant difference among the three groups (p-value: $\leq$ 0.001, R2 : 0.11542). Principal component analysis (PCoA) is depicted in Figure 4B, illustrating the observed variations in microbial community structure between cyclists, weightlifters, and non-athletes.

3.1. Differential Abundance Analysis of Microbial Species

ANCOM-BC (Analysis of Composition of Microbiomes with Bias Correction) is a statistical method employed for the differential abundance analysis of microbial communities.[37] This variant of ANCOM corrects for the bias introduced by the compositional nature of microbiome data. In our study, we utilized ANCOM-BC2 to identify differentially abundant features between groups while effectively controlling for false discovery rate.[37]

In applying ANCOM-BC2, we aimed to pinpoint differentially abundant species among the three groups of interest. This approach offers insights into the specific microbial taxa that drive the observed differences in alpha and beta diversity. The statistical model incorporated control factors such as weight, height, BMI, age, and type of sport. **Table** 3 provides a list of species

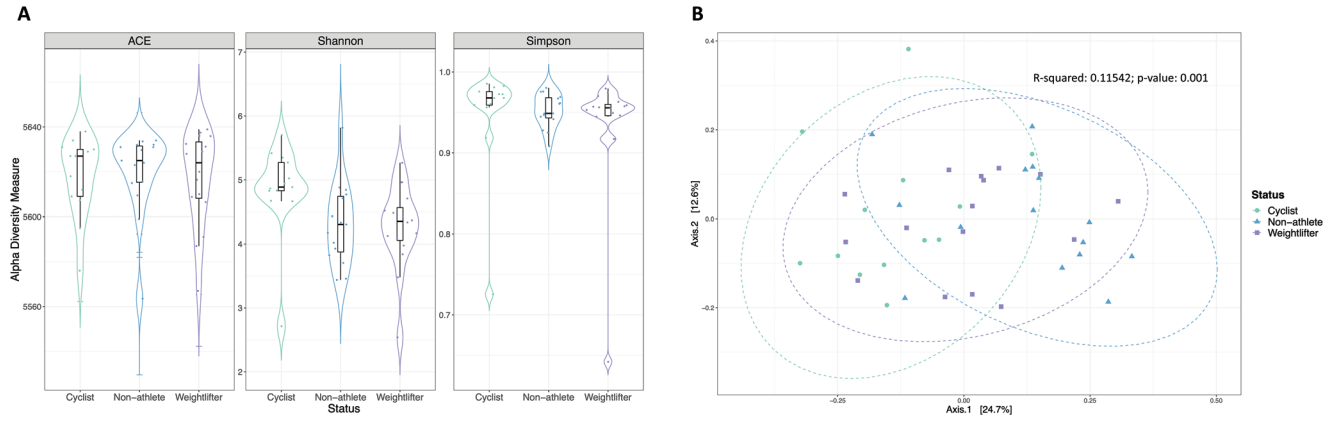

Figure 4. Alpha and Beta diversity of the gut microbiota of Colombian weightlifters, cyclists, and non-athletes subjects. A) Alpha diversity measure. B) Principal Component Analysis (PCoA) Alpha and Beta diversity indexes were computed based on species reads following taxonomical assignment. The disparities were assessed using the Kruskall–Wallis test.

Table 3. Differentially abundant features between cyclists, weightlifters, and non-athletes.

Species	Cyclists vs weightlifters	Cyclists vs non-athletes	Non-athletes vs weightlifters
Agrobacterium tumefaciens	FALSE	FALSE	TRUE
Agrobacterium tumefaciens complex	FALSE	FALSE	TRUE
Clostridium innocuum	TRUE	TRUE	FALSE
Alistipes communis	FALSE	TRUE	FALSE
Alistipes dispar	FALSE	TRUE	FALSE
Alistipes shahii wal 8301	TRUE	TRUE	FALSE
Anaerostipes hadrus	FALSE	TRUE	FALSE
Bacteroidales incertae sedis	TRUE	TRUE	FALSE
Bacteroides caccae	TRUE	TRUE	FALSE
Bacteroides caecimuris	TRUE	TRUE	FALSE
Bacteroides dorei cl03t12c01	FALSE	TRUE	FALSE
Bacteroides fragilis	TRUE	TRUE	FALSE
Bacteroides Fragilis 638r	TRUE	TRUE	FALSE
Bacteroides fragilis ych46	TRUE	TRUE	FALSE
Bacteroides helcogenes p 36–108	TRUE	TRUE	FALSE
Bacteroides heparinolyticus	FALSE	TRUE	FALSE
Bacteroides intestinalis	FALSE	TRUE	FALSE
Bacteroides sp. A1c1	TRUE	TRUE	FALSE
Bacteroides sp. Cacc 737	TRUE	TRUE	FALSE
Bacteroides sp. Cba7301	TRUE	TRUE	FALSE
Bacteroides sp. Hf-5287	TRUE	TRUE	FALSE
Bacteroides sp. Phl 2737	TRUE	TRUE	FALSE
Bacteroides thetaiotaomicron	TRUE	TRUE	FALSE
Bacteroides uniformis	TRUE	TRUE	FALSE
Bacteroides vulgatus atcc 8482	TRUE	TRUE	FALSE
Bacteroides zoogleoformans	FALSE	TRUE	FALSE
Blautia producta	FALSE	TRUE	FALSE
Blautia sp. N6h1-15	FALSE	TRUE	FALSE
Blautia sp. Sc05b48	TRUE	TRUE	FALSE
Clostridioides difficile atcc 43255	FALSE	TRUE	FALSE
Crass-like viruses	FALSE	TRUE	FALSE
Desulfovibrio sp. 86	TRUE	TRUE	FALSE
Eggerthella lenta	FALSE	TRUE	FALSE
Eggerthella lenta dsm 2243	FALSE	TRUE	FALSE
Enterocloster bolteae	FALSE	TRUE	FALSE
Enterocloster clostridioformis	FALSE	TRUE	FALSE
Faecalitalea cylindroides t2-87	FALSE	TRUE	FALSE
Longibaculum sp. Kgmb06250	FALSE	TRUE	TRUE
Massilistercora timonensis	FALSE	TRUE	TRUE
Odoribacter splanchnicus	FALSE	TRUE	FALSE
Parabacteroides distasonis	FALSE	TRUE	FALSE
Parabacteroides distasonis atcc 8503	FALSE	TRUE	FALSE
Phocaeicola dorei	FALSE	TRUE	FALSE
Phocaeicola vulgatus	TRUE	TRUE	FALSE
Unclassified erysipelotrichaceae bacterium gam147	FALSE	TRUE	TRUE
Unclassified parabacteroides sp. Ct06	FALSE	TRUE	FALSE

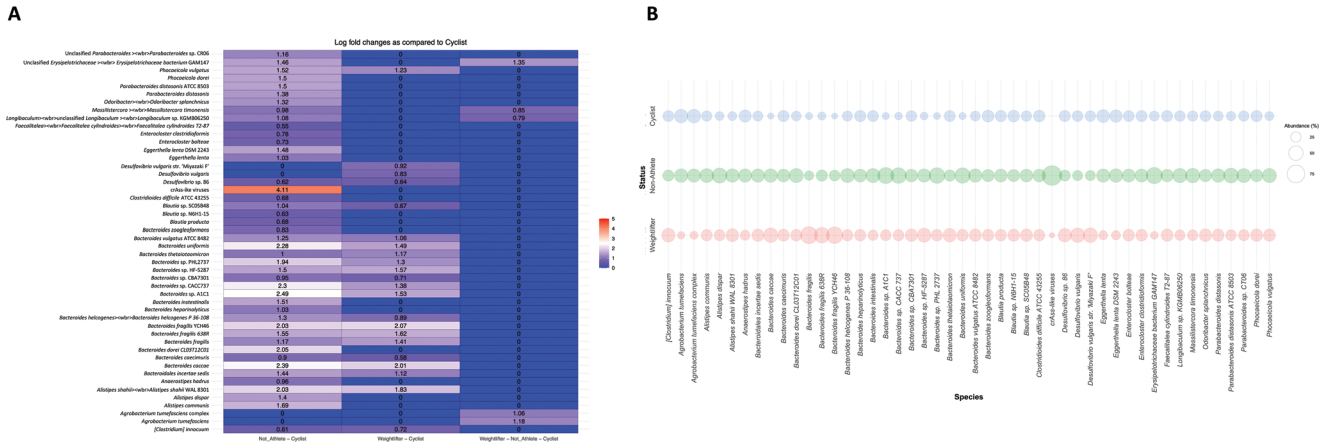

Figure 5. Differentially abundant species between the three groups of interest A) log fold change, comparing microbial species abundance across three groups: cyclists, weightlifters, and non-athletes. The analysis identified 46 species with distinct abundance patterns, revealing significant variations, particularly between cyclists and non-athletes. B) Bubble plot of significant abundant species: This visualization aims to accentuate the taxa that make substantial contributions to the discrepancies in microbial composition, as determined by ANCOM-BC2 analysis.

meeting the criteria to be identified as truly abundant between the groups of interest (p-value: ≤ 0.05; q value: ≤ 0.05). This analysis enhances our understanding of the distinct microbial features contributing to the observed variations in alpha and beta diversity among cyclists, weightlifters, and non-athletes.

The primary results highlight 46 species with distinct abundance features, with significant variations particularly evident in the comparison between cyclists and non-athletes. **Figure** 5A,B identify species that are notably influenced by the differences between weightlifters and cyclists.

To further elucidate these differences, a heatmap (Figure 5A) was generated using log fold change (LFC) abundance. This heatmap specifically illustrates the main distinctions in the comparison of weightlifters versus cyclists and athletes versus non-athletes obtained through ANCOM-BC2. One prominent change is the substantial abundance of crAss-like viruses in non-athlete subjects compared to cyclists. When comparing weightlifters and cyclists, the results show a log fold change (≥1.2) for the following species*: Bacteroides vulgatus ATCC 8482, Bacteroides uniformis, Bacteroides thetaiotaomicron, Bacteroides sp. PHL 2737, Bacteroides sp. HF-5287, Bacteroides sp. CACC 737, Bacteroides sp. A1C1, Bacteroides fragilis 638R, Bacteroides fragilis, Bacteroides caccae, Alistipes shahii WAL 8301, Phocaeicola vulgatus*, and *Bacteroidales incertae sedis*.

To visually compare the percentage of abundance across samples and emphasize taxa significantly contributing to the differences in microbial composition as per the ANCOM-BC2 analysis, a bubble plot was constructed (Figure 5B). This graphical representation aids in highlighting the specific taxa that play a substantial role in differentiating the microbial compositions of weightlifters, cyclists, and non-athletes.

3.2. Correlation Networks Analysis Between Bacterial Archeae and Viral Communities

To delve into the intricate microbial dynamics within athletes and non-athletes, correlation networks were constructed considering bacterial classes, archeal classes, and viral families (**Figure** 6). For this, we calculated Spearman's non-parametric rank-order correlation with Benjamini–Hochberg correction (p-value < 0.05 after FDR correction) and selected only strong correlations ($\rho \leq -0.75$ and $\rho \geq 0.75$).

While positive correlations predominantly characterized the observed connections, indicating complex interactions among families of bacteria and viruses, notable dissimilarities were evident across the networks. Particularly, the cyclist group stood out with a substantial number of correlations between bacterial and archaeal families, suggesting a distinctive microbial interaction pattern in this group of athletes. Cyclists showcased a pronounced connectivity between bacterial and archaeal families, hinting at a specialized microbial interplay associated with their distinct athletic activities.

Building upon these discoveries, non-athletes demonstrated fewer families of viruses involved in the correlation network (only Mimiviridae, which correlates solely with Mollicutes). Conversely, although most correlations were positive, the weightlifters' group stands out as the sole group displaying a negative correlation between the classes Bacteroidia (phylum Bacteroidetes) and Oligoflexia (phylum Pseudomonadota). Additionally, cyclists exhibited a notable linkage between bacterial and archaeal families, indicating a specialized microbial interplay associated with their distinct athletic activities. Such correlation network analysis yields valuable insights into the intricate and unique microbial interactions within these athlete groups.

4. Discussion

This study involved three distinct groups: professional weightlifters, road cyclists, and individuals who were not athletes. It is crucial to highlight the considerable professional experience exhibited by the athletes, with weightlifters averaging 7.44 years ± 2.50 and cyclists averaging 4.85 ± 1.57 years. This extensive athletic experience is a key factor influencing various aspects of the study. Turning our focus to the training regimen,

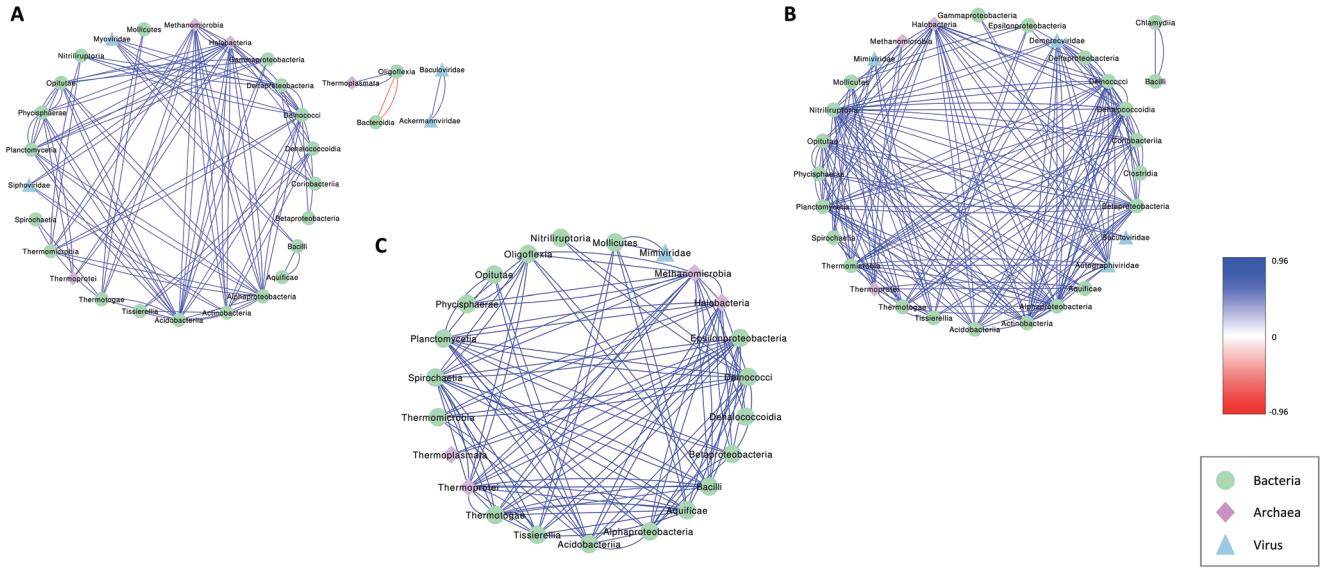

Figure 6. Correlation networks between bacterial classes, archeal classes, and viral families. A) Weightlifters, B) cyclists, C) non-athletes. Correlation networks were created from different taxa levels (class and families). Special features are observed for cyclists, and for weightlifters.

cyclists reported a greater daily training duration compared to weightlifters, while maintaining a consistent training frequency (Table 1). In contrast, weightlifters incorporated sessions specifically tailored to their training routine, concentrating on power and strength exercises performed at an intensity reaching 85% of their maximum strength.

Examining the physical activity levels (PAL) within the non-athletic participants, data collected using the IPAQ revealed a significant prevalence of sedentary behavior, coupled with a moderate-to-low PAL. By comparing these patterns among the various groups, valuable insights can be gained into the distinct lifestyle factors associated with each cohort (Table S2, Supporting Information).

Shifting our attention to the overall composition of the microbiota, it is crucial to explore the broader implications of our findings. Employing MicrobiomeAnalyst 2.0, a univariate analysis was conducted at the family level, revealing notable variations in the relative abundance of microbial families among weightlifters, cyclists, and non-athletes (Table 2, Figure 2A). The Bacteroidaceae family emerged as particularly significant, with a relative abundance of 19.51% among cyclists, markedly lower than the ≥34.05% observed in weightlifters and non-athletes (Figure S2F, Supporting Information). This bacterial family plays a pivotal role in various functions essential for maintaining health, including the regulation of the immune system, food digestion, synthesis of vitamins, and defense against pathogens.[38] This family may hold special importance for athletes requiring efficient energy metabolism during prolonged physical effort, given its capacity to degrade complex carbohydrates.[39] Notably, similar to our findings, some studies have reported lower abundances of this family in endurance athletes,[39,40] while others have found higher abundances,[41] making further analysis necessary to elucidate its functional role in endurance and resistance athletes.

In addition, the abundance of the family Selenomonadaceae, and Spirochaetaceae were different between groups, however, little information is found about these novel microbiota members.[42,43] The Muribaculaceae family stood out as the predominant group among athletes (Figure S2A, Supporting Information), also known as S24-7, this cluster of bacteria forms part of the gut microbiome in mammals, primarily in mice, and is also present in humans, albeit in smaller amounts. The complete understanding of the ecological roles played by these bacteria is still being investigated, mainly due to the limited cultivation and description of family members.[44,45] However, the presence of propionate-producing members from the Muribaculaceae family in mice has been correlated with improved gut health and a longer lifespan.[45,46]

In the context of athletes, propionate, and other short-chain fatty acids (SCFA) may lead to performance enhancements through various mechanisms, including augmented glucose availability, enhanced fatty acid oxidation, and preservation of endogenous glucose levels during physical exertion.[47] Propionate has the potential to meet energy requirements during exercise by acting as a precursor for gluconeogenesis. Furthermore, SCFAs have been shown to regulate skeletal muscle metabolism in a manner similar to exercise-induced changes, promoting the uptake and oxidation of lipids, as well as enhancing glucose uptake.[47] The taxon Prevotellaceae, identified as a distinguishing factor between athletes and non-athletes (Figure 2A, Table 2; Figure S2C, Supporting Information), exhibited a relative abundance of 13.2% in cyclists and 7.4% in weightlifters. This aligns with previous research noting its presence in elite athletes from China,[48] Swiss elite athletes,[49] and a cohort of elite cross-country skiers.[50] Importantly, Petersen et al.[15] discovered a noteworthy correlation between the abundance of *Prevotella* and exercise load in a group of 33 competitive cyclists. This correlation extended to various KEGG pathways, including those associated with amino acid metabolism (e.g., lysine biosynthesis, alanine, aspartate, and glutamate metabolism), carbohydrate metabolism, and the metabolism of cofactors and vitamins, such as vitamin B6.

The utilization of Sankey plots has provided a more profound understanding of microbial composition, offering a visual representation of prevalent bacterial species. *Bacteroidales incertae sedis*, particularly *Bacteroides* spp., emerged as the predominant group across all categories, with *Bacteroides freckles*, *Bacteroides uniformis*, *Faecalibacterium prausnitzii*, and *Phocaeicola vulgatus* exhibiting varying levels of abundance, notably more pronounced in cyclists and weightlifters as shown in Figure 2D–F. These identified bacterial species share distinct metabolic traits. *Bacteroides fragilis*, identified in the gut microbiota of athletes,[20] employs type VI secretion systems featuring novel effector and immunity proteins to counteract human gut *Bacteroidales* species.[51,52] *Bacteroides uniformis*, has been associated with enhanced glucose and lipid metabolism, contributing to a healthier metabolic profile.[53–55] *Faecalibacterium prausnitzii*, plays a role in carbohydrate metabolism, energy production, and lipid metabolism.[56–59] The varied flow of information between groups signifies intricate microbial interactions, highlighting the dynamic nature of the gut microbiota in response to athletic pursuits.

Shifting our attention to Archaea (Figure 2B,C), noticeable differences became apparent, with a significant increase in the relative abundance of families in cyclists compared to weightlifters and non-athletes (Figure S3, Supporting Information). However, the presence of *Methanococcaceae, Methanomicrobiaceae, Methanoregulaceae, Methanosarcinaceae*, and *Haloferacaceae* families has been consistently reported as a recurring pattern within the human gut microbiota.[60–62] While the metabolic function of these archaeal taxa remains poorly understood, they are commonly associated with methane production in the gut.[63,64] Our findings suggest an intriguing correlation between bacterial and archaeal members through correlation networks (Figure 6B), emphasizing the need for further exploration of potential correlations between endurance athletes and methane production. Methanogenic archaea are integral components of the human gut microbiota, playing a role in methane generation through anaerobic fermentation of organic compounds.[62] These microorganisms are associated with the digestion and utilization of dietary components, influencing the efficiency of digestion and overall energy metabolism.[62]

In the context of athletes, such as cyclists, the gut microbiota, including methanogenic archaea, may play a role in influencing energy metabolism and gut health. The production of methane by these archaea may impact both the gut environment and the host's energy balance. However, the specific correlation between methane metabolism and athletic performance, particularly in cycling, remains inadequately explained and necessitates further investigation.

The patterns observed in the cladograms show similarities in weightlifters and non-athletes. Interestingly, *Akkermansia* stood out as a prominent genus across the nonathletic group, although this discovery did not become apparent in single factor analysis or ANCOM-BC2. However, cladograms illustrate *Akkermansia* as a dominant member in the gut microbiota. These results disagree with previous findings,[19,25] where *Akkermansia* (genus) exhibited a notable abundance in the athletic population, conversely, our findings did not show this as a dominant member of the gut microbiota from highly active subjects. Future studies are needed to understand this finding.

We delve into the complexities of microbial community dynamics among cyclists, weightlifters, and non-athletes, employing alpha and beta diversity analyses. These analyses are pivotal in elucidating variations in the microbial community structure, providing insights into the intricate relationships between sport activity and the gut microbiome. Initially, our alpha diversity analysis, as indicated by the Shannon index, did not reveal apparent differences between athletes and non-athletes. However, upon closer inspection, significant variations emerged when comparing cyclists and non-athletes, as well as weightlifters and cyclists (p-value: ≤ 0.001). These findings align with previous observations suggesting shared similarities between weightlifters and non-athletic individuals.[6,8,19,39]

Building upon alpha diversity, beta diversity analysis provided a more comprehensive understanding of the microbial community structure among the three groups. PERMANOVA results indicated a statistically significant difference (p-value: ≤ 0.001, R^2: 0.11542), affirming that the microbial composition varies significantly between cyclists, weightlifters, and non-athletes. The distinctive clustering of microbial communities in the PCoA plot suggests that the type of activity may exert a significant influence on the gut microbiota composition.

Utilizing ANCOM-BC2, we identified 46 species exhibiting notable variations in abundance features. Figure 5A's heatmap illustrates log fold changes (LFC) in abundance, highlighting significant differences between weightlifters and cyclists, as well as athletes versus non-athletes. A noteworthy finding concerns the heightened prevalence of crAss-like viruses in non-athletic individuals compared to cyclists and weightlifters (Figure 5). CrAss-like phages, categorized under Crassvirales, are abundantly present in the human gut microbiota, yet their role remains incompletely understood, subject to ongoing research.[65,66] Recognized for infecting Bacteroidota, particularly targeting Bacteroidaceae within the human gut,[65] crAss-like phages, including lytic infection mechanisms, can modulate bacterial populations, promoting diversity. While some studies suggest a beneficial role for these phages in the human gut, our results imply a potential link between their abundance and athletic performance. However, direct evidence from existing literature on CrAss-like viruses in athletes is lacking, warranting future studies to confirm these findings.

Following our bias-corrected analysis, we have identified several bacterial species exhibiting a log fold change (≥ 1.2) in the comparison between weightlifters and cyclists (Table 3, Figure 5). Notably, some of these species, despite being studied for their metabolic activity and significance in gut health, have not been reported in resistance or endurance athletes. One such species, *Bacteroides vulgatus ATCC 8482*, is recognized for its unique metabolic pathway converting l-galactose into d-tagaturonate. This bacterium is renowned for its ability to break down intricate carbohydrates,[67] metabolize complex carbohydrates, produce butyrate (serving as an energy source for colonocytes), and exhibit anti-inflammatory properties.[67] The presence of *Bacteroides vulgatus ATCC 8482* in weightlifters could hypothetically contribute to supporting gut health and energy metabolism.

Bacteroides uniformis has been the subject of research because of its potential for improving metabolic and immune dysfunction in mice with obesity induced by a high-fat diet. It has been associated with perturbations in colonic microbiota and bile acid

levels.[41,53,54] *Bacteroides thetaiotaomicron* is considered a symbiotic organism of the human gut, with a crucial role in preserving the overall health of the host. In addition to its organic acid fermentation products, it secretes a specific subset of amino acids.[68] The growth of this organism is hindered by acetate and formate,[69] which may potentially aid in protein synthesis and energy metabolism. Lastly, *Bacteroides fragilis 638R* represents a prevalent member of the commensal gut phylum Bacteroidetes. Known for its ability to metabolize various carbohydrates, including host protein glycans, this bacterium produces volatile fatty acids and other carboxylic fermentation products like propionate and lactate.[52,70] The metabolic processes initiated by *Bacteroides fragilis 638R* hold the potential to contribute to energy metabolism in exercise conditions. While these metabolic traits exhibited by *Bacteroides* spp. may have the capacity to enhance the metabolic health, immune function, and energy metabolism of weightlifters or cyclists, a comprehensive understanding of the intricate interplay between these bacteria and the host requires further research. Variables such as diet, body composition, and exercise should be considered in future studies to unravel the full extent of their impact.

Last, we searched to unravel the complex microbial dynamics within athletes and non-athletes, emphasizing the importance of correlation network analysis in discerning nuanced associations in the gut microbiota (Figure 6). By focusing on key bacterial classes, archeal classes, and viral families our study sheds light on the intricate interactions influenced by distinct physical activities. Integrating the Spearman correlation test in our network analysis allowed us to pinpoint significant correlations, revealing predominantly positive correlations and highlighting the intricate interdependencies among bacterial classes and viral families. Notably, the cyclist group displayed a unique pattern with a substantial number of connections between bacterial and archaeal families, hinting at a distinctive microbial interaction pattern influenced by the demands of endurance-based athletic activities. This specialized microbial interplay in cyclists implies potential adaptations supporting energy metabolism and overall gut health during prolonged physical exertion. Our findings echo previous studies that used correlation network analysis to explore interactions in the gut microbiota among athletes.[71,72] In a multicohort study encompassing various sports, distinct network clusters associated with different sports were identified.[72] Correlations between abundant microbiota and functional composition were found, emphasizing the strong association of gut microbiota with physical characteristics, dietary factors, and sport-related features.

This study unveils certain limitations that merit consideration, as they have implications for future research and exploration. A noteworthy limitation is the relatively limited sample size, recognizing its potential implications for the generalizability of the results. It is recognized that the study's broader applicability could be enhanced, and the observed patterns could be more robust by conducting future research with larger cohorts. Moreover, an important consideration involves the imbalance between male and female participants in the control group. The need to address this disparity in future studies is imperative to uphold the integrity of gender-specific analyses and their subsequent interpretations.

Insufficient availability of dietary data poses a significant hindrance to our progress. The study lacks crucial information regarding participants' dietary habits, notably calorie consumption, macronutrient composition, and micronutrient intake. Incorporating dietary data in future investigations could fill this gap, offering a more holistic understanding of the intricate interplay between nutrition and the dynamics of gut microbiota. It's important to recognize that Body Mass Index (BMI) measurements do not provide insights into differential body composition, such as adipose tissue or muscle mass, which can vary significantly among the three groups. Such considerations could also impact the microbiota profile among the groups, underscoring the multifaceted nature of factors influencing gut microbiota dynamics.

Lastly, the study's failure to incorporate metabolic or functional analyses, while solely focusing on taxonomic assignment, highlights another constraint. The thorough investigation of the functional aspects of the gut microbiome offers a promising direction for future research. Engaging in such investigations would enhance our comprehension by offering a more intricate and comprehensive viewpoint on the complex relationship between physical exertion, gut microbiota, and overall well-being.

The findings in this study mark the initial insights into a cohort of Colombian athletes. While past researchers have explored the gut microbiota in highly active and non-active individuals, our approach stands out by incorporating diverse taxonomical and statistical analyses. This allows us to unravel potential links between the gut microbiota's composition (beyond bacteria) and specific activities, such as strength and endurance. Although further investigations are needed, our study represents a step toward characterizing the gut microbiota of Colombian athletes, considering the diverse diets and geographical locations inherent to our region.

5. Conclusion

In conclusion, our study adds valuable insights to the understanding of the gut microbiota in Colombian athletes and non-athletes, unraveling the complex relationship between athletic activity and the human microbiome. Leveraging advanced techniques, such as shotgun metagenomics, we explored the nuances of gut microbiota composition, diversity, and structure in populations engaged in diverse physical activities. Our findings highlight potential microbial signatures linked to specific athletic practices, showcasing substantial differences in the gut microbiota of cyclists, weightlifters, and non-athletes. Using analytical tools like ANCOM-BC2, we identified 46 species with distinct abundance features, emphasizing variations between cyclists and weightlifters. However, it is crucial to acknowledge the study's limitations. The lack of data on dietary habits and individual body composition introduces potentially confounding factors that could impact gut microbiota composition. To bolster our conclusions, future investigations should incorporate these variables into the analysis. While our study reveals intriguing associations between types of sports and gut microbiome patterns, further research is essential to elucidate the intricate relationships between specific physiological aspects related to various activities and the gut microbiome of athletes. This will contribute to a more comprehensive understanding of the dynamic interplay between lifestyle, physiology, and microbial communities within the human body.

6. Experimental Section

Participants: This study was conducted under the principles of the Declaration of Helsinki and was approved by Universidad del Rosario's Research Ethics Committee (CEI-UR) under the approval certificate DVO005 1885-CV1527. All participants provided informed consent to be part of this study. The research, conducted from May to December 2022, included male and female weightlifters from Cali, Colombia, male road cyclists from Paipa, Colombia, and non-athlete subjects from Bogotá, Colombia (Figure S1, Supporting Information). Out of these, 22 weightlifters, 18 cyclists, and 23 non-athlete subjects completed the demographic questionnaire. Finally, 16 weightlifters, 13 cyclists, and 15 non-athletes met the inclusion criteria and were included in this study.

Inclusion and Exclusion Criteria: The inclusion criteria for athletes were individual participants at the professional level engaged in competitive training according to their league calendar, associated with a Colombian sports league. These included: a) Young adults (women and men) (18 to 26 years old); b) Athletes who have been actively training for the past six months; c) Affiliation with a sports league or federation; d) In the pre-competitive stage; e) Been born in Colombia.

The inclusion criteria for non-athletes were: a) Young adults (women and men) (18 to 26 years old); b) Been a Colombian resident; c) Normal body mass index (18–24.9 kg m^{-2}) d) Been born in Colombia

Exclusion criteria included: a) Body mass index (BMI) $\geq$ 25.0 kg m^{-2}, indicating overweight and obesity. b) Use of antibiotics, deworming agents, purgatives, or laxatives within the last 6 months. c) Diagnosis of infectious diseases. d) The presence of non-communicable chronic diseases. e) Diagnosis of metabolic diseases. f) Medical records indicate cardiovascular disease, malignant neoplasm, chronic inflammatory disease, psychiatric disorders, or history of bariatric surgery/liposuction. g) The presence of dyslipidemia, anemia, kidney disease, or smoking.

All participants were required to fill out a demographic questionnaire, providing the following information: date of birth, age, city of residence, city of birth, and gender. Athletes also answered questions about their team, league affiliation, category for upcoming competitions, and recent sports-related injuries in the last three months.

Evaluation of Sport and Physical Activity: The athletes were expressly asked to provide comprehensive details regarding the frequency, volume, and intensity of their training activities in the week prior to the study. Regrettably, this information could not be disclosed due to confidentiality constraints. In contrast, non-athletes were tasked with completing the International Physical Activity Questionnaire (IPAQ), a comprehensive tool designed to quantify the physical activity levels of individuals who did not engage in professional sports[73] (Table S2, Supporting Information). Participants were asked to recall their activities over the past 7 days, detailing the duration and frequency of various physical activities, including walking, moderate-intensity activities, and vigorous-intensity activities. Using standardized protocols, both occupational and leisure-time activities were captured, providing a comprehensive overview of an individual's physical activity behaviors. While athletes supplied information about their training regimen, they did not complete the IPAQ because of privacy policies from local league authorities. The data collection encompassed a range of physical activity domains, such as vigorous-intensity physical activity, moderate-intensity physical activity, walking, and sitting.

Collection of Fecal Samples: To determine the composition and diversity of intestinal microbiota, participants provided a small fecal sample collected at home using a kit supplied by the Center for Microbiology and Biotechnology Research at the University of Rosario (CIMBIUR). The kit included a wide-mouthed container with a collector (small shovel), a pair of disposable gloves, and a small freezer. At the preferred moment of the day, at their place of residence, participants collected a contamination-free sample in the wide-mouthed container, avoiding water or urine. Participants were instructed to introduce the sample immediately into the provided freezer at a temperature of −20 °C until use. A total of 44 samples were collected. All the biological samples were transported to the Microbiology Laboratory at the Universidad del Rosario in Bogotá. Upon arrival at the microbiology laboratory, containers were immediately frozen under −80 °C and did not open until processing. All the samples received treatment for proper storage and subsequent processing to extract the genetic material of the microorganisms contained.

Metagenomic Procedures—DNA Isolation: In order to ensure the inclusion of a broad spectrum of organisms in the analysis, 44 stored stool samples were used for nucleic acid extraction, comprising 16 samples from weightlifters, 13 from cyclists, and 15 from non-athletes. DNA extraction was performed using the commercial QIAamp PowerFecal DNA Kit (Qiagen), designed for the purification of DNA from samples with a high content of inhibitors, such as feces samples and intestinal contents. This kit was widely reported as useful for obtaining high-quality nucleic acids, which allowed their processing on high-throughput sequencing platforms. The quality and concentration of the samples were evaluated using a NanoDrop spectrophotometer and agarose gel electrophoresis.

Metagenomic Procedures—Library Preparation and Sequencing: The library preparation procedure adhered to the specified protocol for the Illumina Nextera XT DNA Library Preparation Kit, ensuring the generation of sequencing-ready libraries. After the normalization of the 44 samples to a concentration of 0.2 ng μL^{-1} per individual, the fragmentation and amplification steps were executed following the kit's guidelines. The quality of the resulting libraries, characterized by fragments of ≈500 bp, was then verified using the Bioanalyzer system. To complete the sequencing process, a certified company was enlisted, with the 44 samples sequenced on the Illumina Hiseq 2500 platform. Notably, the Illumina Nextera XT DNA Library Preparation Kit offered versatile capabilities, preparing libraries suitable for small genomes, such as bacteria, archaea, and viruses, as well as PCR amplicons and plasmids. Key attributes of the kit included a low DNA input requirement, a one-step fragmentation and tagging process.

Metagenomic Procedures—Bioinformatic Analysis: In this study, genomic data was derived from 44 fecal samples (16 weightlifters, 14 cyclists, and 15 non-athletes), each sequenced using the Illumina HiSeq 2500 platform, resulting in an average of 3.2 gigabytes per sample and a cumulative 144.81 gigabytes. The raw sequencing reads underwent quality assessment using FastQC,[77] followed by quality and adapter trimming with Trimmomatic to enhance the reliability of downstream analyses.[78] To eliminate host-related reads, the trimmed data was aligned to the *Homo sapiens* genome using Bowtie2.[79] Taxonomic annotation was executed through read-based approaches, employing Kraken2 with the PlusPF database to classify microbial sequences.[80]

The Pavian package in R was utilized for a thorough analysis and visualization of the metagenomic classification results.[34] Specifically, the Pavian web interface was employed solely for the manipulation of the reports generated from Kraken, offering insights into the taxonomic composition of the fecal samples, and contributing to a holistic understanding of the microbial community structure within the dataset. All graphical representations were created using ggplot2 in R.

Statistical Analysis—Demographic Data: Demographic data from 44 samples were analyzed using the Rcmdr library.[74]

Statistical Analysis—Normality of Data: The Shapiro–Wilk test was applied to assess the normality of continuous data, given the sample size was less than 50 samples. Quantitative variables were selected and grouped by "status" (weightlifter, cyclist, non-athlete) and gender (female, male). Variables analyzed in this section included: age (years), body weight (kg), height (cm), BMI (kg m^{-2}), training frequency (h day^{-1}), training load (days $week^{-1}$), years as a professional athlete. Comparisons were made based on the normality of variables. For instance, non-parametric tests like Kruskal–Wallis or Wilcoxon were used. In the case of height, an independent samples *t*-test or ANOVA for three or more factors was applied. Therefore, the data is presented in medians and IQR.

Statistical Analysis—Microbiota Data Processing: Reads from all the 44 samples were analyzed under the protocol proposed by Chong et al.[35,75,76]

Data Pre-Processing—Integrity Data Check: Clade reads from each domain were extracted using the Pavian package,[34] human and eukaryota data were removed from each domain's dataset. The number of samples processed was 44, any sample was removed in this or any part of the analysis.

Data Pre-Processing—Data Filtering: This step aimed to eliminate low-quality or uninformative features. A minimum count of 4 and a 20% prevalence filter were applied. Variance was measured using inter-quantile range (IQR), and featured with low variance (percentage to remove: 10%) were removed.

Data Pre-Processing—Data Normalization: The data were normalized using total sum scaling without rarefaction or transformation.

Analysis Procedures—Alpha and Beta Diversity Analyses: The aim was to understand the variations in microbial community structure among cyclists, weightlifters, and non-athletes and identify any significant differences. For alpha diversity, ACE, Shannon, and Simpson indexes were calculated across the samples. To detect variations and perform post-hoc analysis, the Kruskal–Wallis test was utilized. Moving to beta diversity, PERMANOVA (Permutational Multivariate Analysis of Variance) was used to calculate the difference among the three groups. Principal Component Analysis (PCoA) was employed for a comprehensive visualization of the relationships.

Analysis Procedures—Core Microbiome Analysis: The core microbiome analysis focused on identifying microbial taxa that were consistently present across all 44 samples within the studied groups (cyclists, weightlifters, and non-athletes). This analysis aimed to reveal the shared microbial communities that persisted regardless of individual variations. To be considered part of the core microbiome, a microbial taxon needed to meet the following criteria: a minimum sample prevalence of 20% and a relative abundance of at least 0.01%.

Analysis Procedures—Single-Factor Statistical Comparisons: Kruskall–Wallis test was employed for statistical comparisons, taking into account the distribution of the data. Post hoc analysis was conducted using the Bonferroni correction to further assess pairwise group differences at the family level, ensuring rigorous control for familywise error rate. The adjusted *p*-value cutoff and statistical method applied were Kruskall–Wallis/Mann Whitney test. This comprehensive analysis was executed at each domain level to identify and validate potential differences across the groups.

Analysis Procedures—Relative Abundance Transformation: The normalized data underwent transformation into relative abundances for presentation and graphical representation. The relative abundance was calculated by taking the counts of each feature and dividing it by the total sum of counts in the respective domain, providing a proportional measure of the composition of microbial taxa within the dataset.

Analysis Procedures—Analysis of Microbial Communities: Cladograms displaying the dominant microbial bacterial classes, archaeal classes, and viral families were correlated with Spearman's non-parametric rank-order correlation with Benjamini–Hochberg correction, considering a p-value $\leq$ 0.05 and a correlation coefficient of −0.75 and 0.75. The correlations were calculated with the psych R package. Subsequently, correlation networks were constructed using igraph, ggraph, and RCy3 R packages and displayed in Cytoscape 3.9.1.l taxa within the three groups were generated by using Graphlan and Kraken reports.[36]

Analysis of Microbial Communities at the Species Level: Lastly, compositional differences in microbial species were assessed using ANCOM-BC2 (analysis of composition of microbiomes with bias correction).[37] In short, clade reads got from Pavian package,[34] phylogenetic information and metadata from participants were transformed into a data frame in the R software, next the ANCOMBC package was used to correct the bias induced by the groups differences through a log-linear regression model, with the aim of identified taxa that were differentially abundant according After data acquisition, the table containing TRUE and FALSE differential abundances resulting from the paired test of ANCOMBC2 was utilized to construct a heat map with log-transformed data. This table contained the log fold change values necessary for heatmap generation.to the variable of interest control factors were weight, height, BMI, age, and type of sport. Finally, a bubble plot was generated to represent the abundance variation of the differentially abundant species across the groups. Compositional analyses and figure construction were all performed using the R v.4.2.1 software, along with vegan, phyloseq, reshape2, tidyverse, and ggplot2 packages (R Core Team, Vienna, Austria).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

J.A., J.D.R., and R.G.-S. conceived the study. J.A., J.D, E.M., D.L., M.P.G., D.M., L.C.-S., A.C., and K.J.Q. recruited the patients, collected the samples, and collected the information of the patients. J.A., M.C., L.V., and M.M. conducted microbiome estimations. J.A. and J.D.R. drafted the manuscript. All authors approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

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CAPÍTULO 3

El capítulo 3 se centra en la compleja interacción entre el microbioma intestinal y el rendimiento deportivo, un área de creciente interés en la investigación actual. El microbioma desempeña un papel fundamental en la modulación del metabolismo del hospedador, y se plantea que los deportistas que participan en disciplinas que requieren distintos sistemas energéticos podrían beneficiarse de los efectos del microbioma en su metabolismo. Este capítulo utiliza un enfoque de integración de ciencias ómicas (metagenómica, metabolómica y lipidómica) para explorar estas dinámicas.

Se reclutaron dos grupos de deportistas colombianos profesionales: levantadores de pesas (n = 16) y ciclistas profesionales (n = 13). El objetivo fue examinar cómo los diferentes sistemas energéticos implicados en sus respectivas disciplinas afectan tanto la composición como la función del microbioma intestinal. Para ello, se llevaron a cabo análisis de metagenómica a partir de muestras fecales, que fueron procesadas utilizando herramientas bioinformáticas como MetaPhlAn 4, StrainPhlAn 4, HUMAnN 3. Además, se realizaron análisis de metabolómica y lipidómica no dirigida en muestras fecales y de plasma.

En este capítulo, el análisis integrativo de metagenómica y metabolómica reveló diferencias importantes en las rutas metabólicas y perfiles de metabolitos entre ciclistas y levantadores de pesas. A través de la metagenómica, se identificaron rutas metabólicas específicas como la biosíntesis de aminoácidos ramificados (valina, leucina e isoleucina), y la biosíntesis de arginina, que fueron más abundantes en ambos grupos de deportistas. Sin embargo, los ciclistas mostraron una mayor diversidad en las rutas relacionadas con el metabolismo de ácidos grasos, lo que podría reflejar la alta demanda energética de actividades de resistencia.

Por otro lado, la metabolómica no dirigida permitió identificar una serie de metabolitos que difieren significativamente entre los grupos bajo estudio. En los levantadores de pesas, se observó un enriquecimiento de metabolitos relacionados con el metabolismo de aminoácidos esenciales y compuestos derivados del metabolismo de nucleótidos, mientras que los ciclistas presentaron mayores concentraciones de metabolitos involucrados en la oxidación de ácidos grasos y la producción de energía a través del ciclo de los ácidos tricarboxílicos (TCA).

Además, el análisis mostró una posible interacción entre el microbioma y el metabolismo del huésped, sugiriendo que ciertos metabolitos producidos por el microbioma podrían estar influyendo en las rutas metabólicas del atleta. Un hallazgo clave fue la correlación entre ciertos metabolitos lipídicos y la presencia de rutas microbianas relacionadas con la biosíntesis de lípidos. Los ciclistas, en particular, mostraron un perfil lipídico más diverso, lo que sugiere que el tipo de ejercicio podría modular no solo el microbioma, sino también el metabolismo lipídico a nivel sistémico.

Finalmente, el análisis de redes entre los metabolitos y las rutas microbianas destacó conexiones entre rutas bacterianas específicas, como la biosíntesis de ácidos grasos de

cadena larga y la producción de metabolitos como el propionato y acetato, que podrían influir en la eficiencia energética y la adaptación al ejercicio en estos atletas. Aunque las conexiones ilustradas son hipotéticas y requieren validación adicional, estos resultados subrayan el potencial de la integración multiómica para descubrir relaciones complejas entre el microbioma y el metabolismo del huésped en el contexto del rendimiento físico. Los resultados a mayor detalle pueden ser encontrados en el siguiente artículo:

- **Artículo 4 (Sometido a Sports Medicine):** **Aya V**, Pardo D, Vega LC, Cala MP, Ramírez JD. Integrating Metagenomics and Metabolomics to Study the Gut Microbiome and Host Relationship in Sports Across Different Energy Systems.

CAPÍTULO 3: Interacción microbioma - hospedero en sujetos altamente entrenados: una aproximación desde las ciencias ómicas.

Sports Medicine

Integrating Metagenomics and Metabolomics to Study the Gut Microbiome and Host Relationship in Sports Across Different Energy Systems

--Manuscript Draft--

Manuscript Number:	
Full Title:	Integrating Metagenomics and Metabolomics to Study the Gut Microbiome and Host Relationship in Sports Across Different Energy Systems
Article Type:	Original Research Article
Funding Information:	Universidad del Rosario (Internal funds) — Prof. Juan David Ramirez
Abstract:	The gut microbiota plays a crucial role in modulating host metabolism, particularly in athletes engaged in sports requiring varying energy systems. This study aims to elucidate the intricate relationship between gut microbiota composition and host metabolic responses in athletes by employing integrative omics approaches. Two groups of Colombian athletes were recruited: elite weightlifting athletes (WA; n = 16) and elite cyclist athletes (CA; n = 13). Both groups, composed of athletes from professional Colombian leagues, were measured one month prior to an international competition. Metagenomics analysis was performed using fecal samples, processed through bioBakery tools to assess the microbial composition. Metabolomics analysis was conducted on both fecal and plasma samples using advanced chromatography and high-resolution mass spectrometry techniques. Lipidomics analysis was also carried out on plasma samples to explore lipid profiles. Metagenomic features, including relative abundance and pathway analysis using MetaCyc, were combined with metabolomic and lipidomic data. This comprehensive data was then subjected to data acquisition, statistical analysis, and visualization techniques to identify significant metabolic pathways and features related to athletic performance, culminating in the identification of key metabolites and pathways. Our metagenomic analysis revealed the presence of specific pathways, including L-arginine biosynthesis III (via N-acetyl-L-citrulline) and fatty acid biosynthesis initiation (type II). Furthermore, our integrative analysis highlighted the enrichment of key metabolic pathways, including phenylalanine, tyrosine, and tryptophan biosynthesis, arginine biosynthesis, valine, leucine, and isoleucine biosynthesis, histidine metabolism, and folate biosynthesis. Additionally, plasma metabolomics and lipidomics demonstrated a clear separation through multivariate models, revealing the abundance of diverse significant metabolites across weightlifters and cyclists. Mainly, the metabolic pathways driving the separation across groups were related to lipids, including upregulated pathways involved in lipid droplet formation, lipid storage, and glycolipid synthesis. Additionally, the analysis revealed a notable abundance of carnitines, along with various classes of metabolites such as amino acids and glycerolipids. These findings suggest a distinct interplay between gut microbiota and host metabolism tailored to different energy systems in sports, providing a foundation for personalized interventions aimed at optimizing athletic performance through targeted modulation of gut microbiota.
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Editor-in-Chief
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Dear Editor,

I am pleased to submit our manuscript titled "Integrating Metagenomics and Metabolomics to Study the Gut Microbiome and Host Relationship in Sports Across Different Energy Systems" for consideration for publication in Sports Medicine. This work provides novel insights into the intricate relationship between gut microbiota composition and host metabolic responses in athletes from distinct disciplines by employing cutting-edge integrative omics approaches.

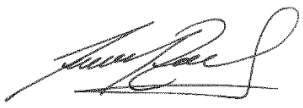
In this study, we recruited two groups of elite Colombian athletes, including weightlifters (WA; n = 16) and cyclists (CA; n = 13), to investigate the interplay between the gut microbiota and host metabolism. Our comprehensive analysis utilized metagenomics, metabolomics, and lipidomics on fecal and plasma samples to identify key metabolic pathways related to athletic performance, particularly in relation to the differing energy systems of weightlifting and cycling. We identified several enriched metabolic pathways, including those involved in amino acid biosynthesis and lipid metabolism, which we believe are critical to optimizing athletic performance through targeted microbiota modulation.

This research highlights the potential of personalized microbiota-based interventions to enhance athletic performance and presents the first integrative study of its kind in professional Colombian athletes. Given the growing interest in the role of the gut microbiome in sports performance, we believe that this work will be of great interest to the readers of Sports Medicine.

We confirm that the manuscript has not been previously published and is not under consideration for publication elsewhere. All authors have read and approved the final version of the manuscript, and there are no conflicts of interest to disclose.

Thank you for your consideration of our work. We look forward to your response and the opportunity to contribute to the ongoing scientific discourse in the field of sports medicine.

Sincerely,



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Integrating Metagenomics and Metabolomics to Study the Gut Microbiome and Host Relationship in Sports Across Different Energy Systems

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Abstract

Background: The gut microbiota plays a crucial role in modulating host metabolism, particularly in athletes engaged in sports requiring varying energy systems.

Objective: This study aims to elucidate the intricate relationship between gut microbiota composition and host metabolic responses in athletes by employing integrative omics approaches.

Methods: Two groups of Colombian athletes were recruited: elite weightlifting athletes (WA; n = 16) and elite cyclist athletes (CA; n = 13). Both groups, composed of athletes from professional Colombian leagues, were measured one month prior to an international competition. Metagenomics analysis was performed using fecal samples, processed through bioBakery tools to assess the microbial composition. Metabolomics analysis was conducted on both fecal and plasma samples using advanced chromatography and high-resolution mass spectrometry techniques. Lipidomics analysis was also carried out on plasma samples to explore lipid profiles. Metagenomic features, including relative abundance and pathway analysis using MetaCyc, were combined with metabolomic and lipidomic data. This comprehensive data was then subjected to data acquisition, statistical analysis, and visualization techniques to identify significant metabolic pathways and features related to athletic performance, culminating in the identification of key metabolites and pathways.

Results: Our metagenomic analysis revealed the presence of specific pathways, including L-arginine biosynthesis III (via N-acetyl-L-citrulline) and fatty acid biosynthesis initiation (type II). Furthermore, our integrative analysis highlighted the enrichment of key metabolic pathways, including phenylalanine, tyrosine, and tryptophan biosynthesis, arginine biosynthesis, valine, leucine, and isoleucine biosynthesis, histidine metabolism, and folate biosynthesis. Additionally, plasma metabolomics and lipidomics demonstrated a clear separation through multivariate models, revealing the abundance of diverse significant metabolites across weightlifters and cyclists. Mainly, the metabolic pathways driving the separation across groups were related to lipids, including upregulated pathways involved in lipid droplet formation, lipid storage, and glycolipid synthesis. Additionally, the analysis revealed a notable abundance of carnitines, along with various classes of metabolites such as amino acids and glycerolipids.

Conclusion: These findings suggest a distinct interplay between gut microbiota and host metabolism tailored to different energy systems in sports, providing a foundation for personalized interventions aimed at optimizing athletic performance through targeted modulation of gut microbiota.

1. Introduction

The gut microbiota of physically active individuals has been thoroughly described, exhibiting a greater prevalence of beneficial microorganisms [1–3], specifically the gut microbiota from athletes has been characterized by a higher prevalence of butyrate-producing bacteria such as *Eubacterium rectale* [4], *Akkermansia muciniphila* [5], *Eubacterium hallii* [6,7], *Faecalibacterium prausnitzi* [8,9], among others [10]. Metagenomic data from highly trained subjects, shows the presence of numerous bacterial families involved in crucial metabolic functions [11]. Recent research showed that exercise led to elevated levels of lysophosphatidylcholine species synthetized by gut microbiota, leading an enhanced lipid oxidation, and reduced activity in metabolic pathways associated with oxidative stress [12].

Most of the research in this field has focused on endurance training, particularly aerobic exercise [13,14], which is key for energy production via the oxidative system [1,15]. Endurance athletes, like cyclists and runners, demonstrate exceptional cardiovascular fitness and enhanced oxidative metabolism. While many studies have examined the microbiome's role in these activities [16–18], the effects of strength and other exercise forms on gut microbiota remain underexplored. Including

athletes from various backgrounds could reveal the gut microbiome's diverse functional capabilities and its reciprocal relationship with exercise. Traditionally, exercise metabolism has been divided into glycolytic and oxidative pathways, reflecting the energy systems engaged during different exercise intensities and durations. However, recent research suggests that gut microbiota's metabolic functions may play a significant role in supporting athletes' overall performance in physically demanding activities [19].

In order to comprehend the intricate relationship between sports activities and their effects on the gut microbiome, the utilization of comprehensive multi-omics analyses proves to be a robust investigative toolset [20–23]. By incorporating data from various approaches, such as metagenomics, transcriptomics, proteomics, metabolomics, and lipidomics, it is possible to draw conclusions that provide valuable insights into the molecular mechanisms behind athletic adaptation [19,24]. Among these approaches, metabolomic analysis stands out for its ability to provide a detailed snapshot of the metabolites and their pathways, shedding light on the functional implications of microbiota changes induced by physical activity [25].

The usage of metabolomics in sports fields has grown considerably in the past years due to representing a comprehensive approach to detecting metabolomic changes in response to different physical activity stimuli [26] and the advantage that metabolomic methods offer in order to high throughput quantification of hundreds of metabolites in a single sample such as blood, urine, saliva, or feces [22,27]. Moreover, recent research highlights the correlation between gut microbiota, exercise, and the metabolome. Specifically, the presence of *Butyricicoccus* genus has been linked to higher levels of beneficial HDL cholesterol components and larger HDL particle size. Conversely, a decrease in the *Ruminococcus* group among athletes correlates with higher total cholesterol and LDL levels. These results suggests that elite athletes exhibit a more favorable lipid profile due to the presence of specific gut microbes associated with better overall health [16].

The present study investigates the metabolic dynamics of Colombian elite athletes, specifically professional cyclists, and weightlifters, using a multi-omics integration under a knowledge-driven approach. By integrating metagenomic and metabolomic data, this study seeks to elucidate the interplay between gut microbiota, host metabolism, and athletic performance. Ultimately, it aims to uncover novel insights into how the gut microbiota influences systemic metabolism and its implications for enhancing athletic performance.

2. Materials and methods

2.1 Participants

Two groups of Colombian athletes, elite weightlifting athletes (WA; n = 16, age: 29.31 ± 9.8 years, weight: 68.31 ± 12.9 kg, height: 167 ± 6.5 cm, BMI: 26.5 ± 3.2 kg/m^2), and elite cyclist athletes (CA; n = 13, age: 20.63 ± 1.12 years, weight: 59.77 ± 6.57 kg, height: 169.7 ± 7.8 cm, BMI: 20.72 ± 1.5 kg/m^2), were recruited for this study. Both groups included athletes of the professional Colombian leagues of weightlifting (men = 8; women = 9), practicing competitive sports for 7.5 ± 2.5 years; and cycling (men = 13), practicing competitive sports 5.0 ± 1.6 years at national and international level. Both elite athletes' groups were measured one month prior to an international competition. The inclusion/exclusion criteria applied in this study are detailed in our previous publication [19]. In brief, professional-level athletes engaged in competitive training according to their league's schedule and affiliated with a Colombian sports league were invited to participated in this study. Specific requirements included: a) Adults (men and women) aged 18 to 45; b) Athletes who have been actively training for at least the past six months; c) Affiliation with a recognized sports league or federation; d) Currently in the pre-competitive stage; and e) Born in Colombia.

Exclusion criteria included: a) Use of antibiotics, deworming agents, purgatives, or laxatives in the past six months; b) Diagnosis of infectious diseases; c) Presence of non-communicable chronic diseases; d) Diagnosis of metabolic diseases; e) Medical history of cardiovascular disease, malignant neoplasm, chronic inflammatory disease, psychiatric disorders; f) Presence of dyslipidemia, anemia, kidney disease, or smoking. Figure 1 presents a comprehensive overview of the methodological workflow used to study the gut microbiota and host metabolism in athletes.

All participants attended to the laboratory appointment under similar conditions: 8 hours of fasting and not having trained 24 hours prior to attending the test center. We extracted blood from all the participants, collected plasma, and stored it at -80°C until analysis. Each participant provided fecal samples, which were stored at -80°C for further analysis. The collection and storage procedures for fecal samples followed established protocols [28] to ensure sample integrity and minimize contamination. The researchers labeled all samples with unique identifiers to maintain anonymity and track individual data throughout the study period [29].

2.2 Gut Microbiome Analysis Through Metagenomic Analysis

2.2.1 DNA extraction and quality control

DNA extraction for compositional and functional analysis from microbiome dataset were developed to ensure representation across a diverse range of organisms, we utilized 29 stored stool samples. These samples included 16 from weightlifters, 13 from cyclists. The DNA extraction process employed the QIAamp PowerFecal DNA Kit by Qiagen, specifically designed for purifying DNA from samples rich in inhibitors (such as feces and intestinal contents). To determine purity and ensure precise quantification, a Nanodrop Kit (Implen, CA, USA) and a Qubit® 2.0 fluorometer (Life Technologies, CA, USA) were employed, respectively.

2.2.2 Library preparation and sequencing

The protocol outlined for the Illumina Nextera XT DNA Library Preparation Kit was meticulously followed. Initially, samples were normalized to a concentration of 0.2 ng/μL per individual. This was followed by fragmentation and amplification steps. An index code was incorporated into the primer to differentiate various samples in the sequence data. After verifying the quality of the library, all samples were subjected to paired-end sequencing using the Illumina HiSeq 2500 platform by the certified company. Sequencing depth was maintained at 2 GB for each sample, ensuring comprehensive coverage of genetic material. This depth allowed for robust analysis and identification of functional features such as gene family abundances.

2.2.3 Quality Control and Data Processing for metagenomic analysis

The raw sequencing reads obtained from the Illumina platform underwent a series of quality control steps. First, quality assessment was performed using FastQC. Next, quality and adapter trimming were carried out using Trimmomatic to enhance the reliability of downstream analyses. To remove host-related reads, the trimmed data was aligned to the *Homo sapiens* genome using Bowtie2. The resulting reads were then utilized to perform taxonomical and functional analyses using the BioBakery workflow [30], which includes a suite of tools designed for comprehensive microbial community profiling and functional annotation [30].

2.2.4 Taxonomic profiling using MetaPhlAn4 and StrainPhlAn4 methods

Clean reads obtained from metagenomic samples, consisting of 16 samples from weightlifters and 13 samples from cyclists, underwent taxonomic profiling using MetaPhlAn4 [31] and StrainPhlAn4 [32]. MetaPhlAn 4 utilizes a database of clade-specific marker genes derived from known microbial genomes. This approach allows for the identification and quantification of microbial taxa present in the samples based on the alignment of cleaned reads against these markers. The relative abundance of each taxon in the metagenomic samples was determined from the alignment results generated by MetaPhlAn4 [33].

StrainPhlAn4 extends this analysis by performing strain-level profiling of microbial communities. It utilizes single nucleotide variants (SNVs) in core genes to differentiate strains within taxonomic groups identified by MetaPhlAn4 [34]. This provides a more detailed characterization of microbial populations, elucidating strain-level differences between samples. The taxonomic and strain-level profiles obtained from MetaPhlAn 4 and StrainPhlAn 4 were further analyzed statistically to assess differences in microbial composition and strain diversity between weightlifters and cyclists. Statistical significance of differences in microbial composition and strain diversity was evaluated using non-parametric tests due to the distribution of the data. MaAsLin 2.0 was employed to perform multivariate analysis through a lineal model [35] .

To ensure the reliability of taxonomic and strain-level assignments, outputs were validated against established databases of microbial genomes (NCBI). Quality control measures were implemented throughout the analysis pipeline to validate the accuracy of taxonomic and strain-level profiling and to minimize potential biases. Taxonomic and strain-level profiles were visualized using Microbiome analyst 2.0 [36] and R-based tools to illustrate the microbial community structure and strain diversity across samples.

2.2.5 Functional profiling using HumanN4 methods

Following taxonomic profiling, HUMAnN 4 was employed to perform functional annotation of the 29 samples [30]. It maps the cleaned reads against a comprehensive database of reference sequences, including microbial genomes and gene families, to identify and quantify the presence of functional pathways and gene functions within the microbial community. Functional profiling using HUMAnN4 revealed insights into microbial metabolic pathways, providing quantitative

assessments of specific functions within the gut microbiota of both athlete groups. MaAsLin 2.0 facilitated differential analysis, pinpointing metabolic pathways exhibiting significant differences between weightlifters and cyclists. Further data analysis involved Microbiome Analyst 2.0 for gene abundance analysis, clustering, and univariate analysis, offering a comprehensive view of the gut microbiome's functional composition and associated patterns. MetaCyc reference databases was used to provided additional insights into metabolic pathways [37].

2.3 Untargeted Metabolomic and Lipidomic Analysis For Fecal and Plasma Samples

In this study, we employed untargeted metabolomic and lipidomic analyses to investigate the metabolic profiles of fecal and plasma samples from weightlifters (16 samples) and cyclists (13 samples). Plasma samples specifically underwent lipidomic analysis to characterize lipid profiles. Various analytical platforms, including Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS), were utilized to comprehensively analyze the metabolites present in these samples. This approach allows exploration of metabolic pathways and lipid compositions, providing insights into metabolic differences between athletes from different disciplines.

2.3.1 Metabolomic analysis for fecal samples by LC-MS and GC-MS Analysis

Sample preparation for fecal metabolomic analysis by Reverse Phase Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (RP-LC/MS-QTOF) began with lyophilization to remove moisture content, followed by precise weighing. Each sample was allocated for subsequent processing, extraction, and identification using an optimized protocol tailored for untargeted metabolomic analysis [38].

Fecal samples (50 mg) were weighed and mixed with 1000 µL of MeOH, vortexed for 15 minutes, and then sonicated for 15 minutes. After another vortex step (5 minutes), samples were centrifuged at 16000 rpm, 4°C for 10 minutes. Subsequently, 100 µL of the extract was collected for further analysis by LC-QTOF-MS.

Samples were analyzed using an Agilent Technologies 1260 Liquid Chromatography system coupled to a quadrupole time-of-flight Q-TOF 6545 mass analyzer with electrospray ionization. A 1 µL aliquot of each sample was injected onto a C_{18} column (InfinityLab Poroshell 120 EC-C_{18} 100 x 2.1 mm, 1.9 µm) at 40°C, using a gradient elution composed of 0.1% (v/v) formic acid in

Milli-Q water (Phase A) and 0.1% (v/v) formic acid in acetonitrile (Phase B) at a constant flow rate of 0.4 mL/min. Mass spectrometry detection was conducted in positive electrospray ionization (ESI) mode, scanning from 50 to 1100 *m/z* in both full scan and MS/MS modes. Throughout the analysis, mass correction was performed using two reference masses: *m/z* 121.0509 ($C_5H_4N_4$) and *m/z* 922.0098 ($C_{18}H_{18}O_6N_3P_3F_{24}$).

From the extracts prepared for RP-LC/MS-QTOF analysis, 20 µL were dried in a SpeedVac for 1 hour. Then, 10 µL of *O*-methoxime in pyridine (15 mg/mL) were added, vortexed for 5 minutes, and incubated in the dark at room temperature for 16 hours. Silylation was performed by adding 10 µL of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), followed by vortexing for 5 minutes and incubation at 70°C for 1 hour. After cooling to room temperature, 200 µL of methyl stearate (5 mg/L) were added as an internal standard. Data acquisition was performed on an Agilent Technologies 7890B GC coupled with an Agilent Technologies 7250 GC/Q-TOF mass spectrometer. A 1 µL aliquot of derivatized sample was injected with a split ratio of 30:1 onto an HP-5MS column (30 m, 0.25 mm, 0.25 µm) (Agilent Technologies) at a constant flow rate of 0.7 mL/min. The oven temperature was programmed from 60°C (1 minute) at 10°C/min to 325°C (10 minutes). Mass spectra were recorded at 70 eV in full scan mode with values ranging from 50 to 600 *m/z*. The transfer line, ion source, and quadrupole temperatures were maintained at 280°C, 230°C, and 150°C, respectively.

2.3.2 Metabolomic analysis of plasma samples by GC-QTOF-MS and LC-QTOF-MS.

200 µL of plasma was mixed with 600 µL of cold MeOH, vortexed, and stored at -20 °C for 20 minutes before centrifugation. For GC-QTOF-MS analysis, 50 µL of plasma extract preparation was dried and subjected to derivatization following the previously described procedure.

The LC-QTOF-MS analysis of plasma samples was conducted using an Agilent Technologies 1260 Liquid Chromatography system coupled to a 6545 Q-TOF time-of-flight quadrupole mass analyzer with electrospray ionization, following the same methodology as previously described for fecal samples.

2.3.3 Lipidomic Analysis of Plasma Samples by LC-QTOF-MS

The process involved extracting 100 µL of plasma and then combining it with 350 µL of cold MeOH (-20°C) and an additional 350 µL of MTBE. Following that, they were subjected to

vortexing for 5 minutes and subsequently centrifuged at 13000 rpm and a temperature of 20°C for a period of 10 minutes. For further analysis, a total of 100 µL of the supernatant was transferred into an Eppendorf tube.

The lipidomic analysis of plasma samples was performed using an Agilent Technologies 1260 Liquid Chromatography system, which was coupled to a 6545 Q-TOF time-of-flight quadrupole mass analyzer with electrospray ionization. A volume of 1 µL of the extracts was introduced into a C_{18} column (InfinityLab Poroshell 120 100 x 3.0 mm, 2.7 µm) maintained at a temperature of 50 °C. The elution process followed a gradient composition consisting of 10mM ammonium acetate in a ratio of 90:10 (ACN:H_2O) (Phase A) and 10mM ammonium acetate in 20:30:50 (ACN:MeOH:IPA) (Phase B) with a constant flow of 0.4 mL/min. Mass spectrometry detection was conducted using positive electrospray ionization (ESI) mode in both full scan and MS/MS modes, covering the mass range of 40 to 2000 *m/z*. In the course of the analysis, the reference masses utilized for mass correction were: *m/z* 121.0509 ($C_5H_4N_4$), *m/z* 922.0098 ($C_{18}H_{18}O_6N_3P_3F_{24}$).

2.3.4 Quality control of metabolomic and lipidomics analyses

Quality assurance and control practices in metabolomic analyses by MS followed the recommendations of the international mQACC consortium [39]. These practices included the analysis of quality control (QC) samples, reference standards, and blanks. The QC samples were prepared by pooling equal volumes of the samples to be analyzed and were extracted and analyzed in the same manner as the study samples. To assess the reproducibility and stability of the analytical platforms used, QC samples were analyzed before the analysis sequence to balance the chromatographic system and were injected every five samples throughout the sequence.

2.3.5 Data processing and Statistical Analysis from metabolomic and lipidomic data

The raw data from the LC-QTOF-MS system was processed using Agilent MassHunter Profinder B.10.0 software for deconvolution, alignment, and integration. For the GC-QTOF-MS data, these steps were carried out with Agilent Unknowns Analysis B.10.0, MassProfiler Professional B.15.0, and Agilent Mass Hunter Quantitative Analysis B.10.0. Subsequently, the data from all platforms were thoroughly analyzed. A presence and reproducibility filter were applied, retaining only

metabolites found in at least 80% of the samples within the same group and exhibiting a coefficient of variation (CV, %) below 20% in QC samples for LC data (30% for GC data). These filtered metabolites were then selected for statistical analysis.

To identify molecular features with statistically significant differences between weightlifters and cyclists, both univariate (UVA) and multivariate (MVA) statistical analyses were performed using the MetaboAnalyst server. For the UVA analysis, p-values were computed using nonparametric tests. In the MVA analysis, principal component analysis (PCA) was initially employed as an unsupervised method to evaluate data quality and sample distribution. This was followed by the application of supervised orthogonal partial least squares discriminant analysis (OPLS-DA) models to identify the molecular features driving group separation. The effectiveness and precision of the OPLS-DA models were assessed using R^2, Q^2, permutation tests, and cross-validation analysis of variance. Statistically significant features were selected based on the following criteria: (1) UVA—p-value < 0.05 and (2) MVA—variance important in projection (VIP) > 1.

2.3.6 Metabolites identification

To annotate significant features from liquid chromatography, a variety of parameters were employed. These included confirming retention times and the potential for adduct formation, comparing high-resolution mass data with database entries using the CEU Mass Mediator tool (http://ceumass.eps.uspceu.es, accessed on 2023), and deriving theoretical formulas based on isotopic distributions. MS/MS data were cross-referenced with spectra in MS-DIAL 4.80 (http://prime.psc.riken.jp/compms/msdial/main.html) and Lipid Annotator software v10.0. Manual interpretation of MS/MS spectra was also conducted. For GC analysis, compound identification was achieved by comparing mass spectra and FAMES retention indices with entries in the Fiehn GC-MS Metabolomics RTL (Retention Time Locked) Library 2013 (https://pubs.acs.org/doi/abs/10.1021/ac9019522). Finally, identification levels were assigned for each platform according to the guidelines provided by the Metabolomics Standards Initiative as described by Blaženović et al. (2018). The lowest level of annotation was achieved with a match to the exact mass (4), followed by confirmation of the molecular formula (3), identification of specific compound fragment signals (2), and, ultimately, confirmation at the standard level (1). [40].

2.4 Integrative Analysis of Gut Microbiome Functional Profiling

In this study, state-of-the-art bioinformatic tools were employed to investigate the functional potential of the gut microbiome in weightlifters and cyclists [30,41–43]. An integrative analysis of the gut microbiome and hypothetical metabolites, reviewed from literature, produced by the gut microbiota was used to create a multi-omics integration under a knowledge-driven approach, following the methodology proposed by Ewald and collaborators (Web-based multi-omics integration using the Analyst software suite) [42]. The Analyst software suite, specifically OmicsNet 2.0, was utilized for this purpose [41].

The model construction followed the approach described by Ewald et al., wherein each omics data type was analyzed separately. After statistical treatment, significant species identified by MetaPhlAn4 and metabolites identified from fecal and plasma metabolomic analysis were selected to create an integrative network. This multi-omics integration provided a comprehensive view of the interactions between the gut microbiome and metabolomic profiles, enabling a deeper understanding of the functional dynamics in weightlifters and cyclists.

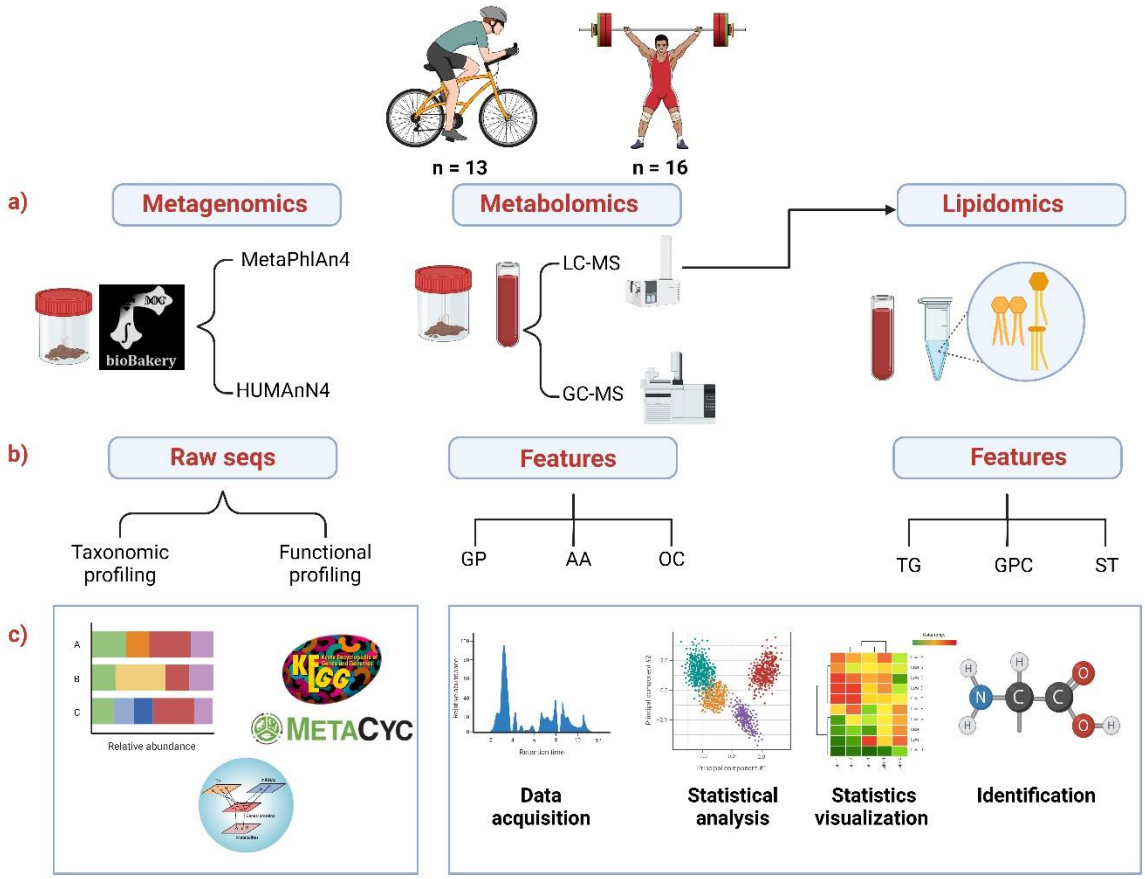


Fig 1. Overview of Integrative Omics Workflow for Studying Gut Microbiota and Host Metabolism in Athletes, summarizes the multi-step process from sample collection to data integration, providing a holistic view of the experimental approach used to investigate the interplay between gut microbiota and host metabolism in athletes. **(a) Sample Collection and Analytical Techniques**, this section illustrates the different omics approaches employed in the study. **(b) Data Processing and Feature Extraction** outlines the transformation of raw sequences and data into usable features for analysis. In metagenomics, raw sequence data was generated, while in metabolomics and lipidomics, feature extraction was performed to identify specific metabolites and lipids from the chromatographic data. **(c) Data Integration and Analysis** showcases the integration of different omics data. The artwork was created using Biorender. **GP:** Glycerophospholipid; **AA:** Amino acids; **OC:** Organic compounds; **TG:** Triglycerides; **GPC:** Glycerophosphocholine; **ST:** Sterols.

3 Results

3.1 Species and strain taxonomic profiling from samples of Colombian weightlifters and cyclists

In this study, we aimed to further characterize the gut microbiota of Colombian elite athletes—weightlifting athletes (WA; n = 16) and cyclist athletes (CA; n = 13)—using a functional analysis approach with the biobakery3 suite, specifically MetaPhlAn4 and StrainPhlAn4 [30,33,34]. This builds upon our previous work, where we conducted a taxonomic profile analysis using Kraken assignment on the same fecal samples, which focused on identifying archaeal and bacterial signatures in these athletes compared to non-athletes [44].

For this analysis, we employed the MetaPhlAn4.0 workflow to obtain compositional profiles of the gut microbiota [33], moving beyond mere taxonomic classification to explore functional potential. We applied a low abundance filter (10%) and a median abundance value filter, refining the results to approximately 300 species. An abundance analysis was then performed to identify the most prevalent species across both groups of athletes, highlighting key microbial differences linked to their distinct physical activities. This complementary approach enables a more comprehensive understanding of the gut microbiota in elite athletes by integrating both taxonomic and functional profiles.

The identified species are visually represented in Figure 2A and detailed further in Supplementary Table 1. The most abundant species identified in both athlete groups include members of the genus *Bacteroides*, *Eubacterium*, *Prevotella*, *Firmicutes*, *Alistipes* and *Faecalibacterium.*

Statistical analysis was performed to identify important differences between the two groups of athletes: weightlifters (WA) and cyclists (CA). Multiple Linear Regression with Covariate Adjustment using MaAsLin2.0, was implemented, however we did not find any significant associations when considering both p-value and false discovery rate (FDR) corrections. Single factor analysis (Supplementary Table 2), was used to identify specific features that were significant in differentiating WA and CA: *Prevotella_sp_CAG_386* (p-value: 0.0002), *Bacteroides_fragilis* (p-value: 0.001), *Alistipes_putredinis* (p-value: 0.004), *Prevotella_sp_CAG_873* (p-value: 0.006), *Bacteroides_uniformis* (p-value: 0.006), *Bacteroides_caccae* (p-value: 0.01),

Succinatimonas_sp_CAG_777 (p-value: 0.04), *Eubacterium_rectale* (p-value: 0.04), *Parabacteroides_merdae* (p-value: 0.04).

Following the initial assessment of microbial community composition, we identified key species of interest for further analysis. Among the species identified, *Bacteroides caccae* (Figure 2B), *Bacteroides finegoldi I* (Figure 2C), and *Prevotella copri* (Figure 2D) emerged as significant due to their prominence in the samples and potential implications for host metabolism. These species were selected for detailed strain-level characterization using StrainPhlAn4 [34]. The subsequent distance-based analysis aimed to elucidate the strain diversity and genetic relationships within these species, providing deeper insights into their roles and potential adaptations in the two groups of athletes. As noted in figure 2, analysis produced distance trees, where the X-axis represents cladistic distance rather than traditional phylogenetic trees.

Sequences from the samples were aligned, and a strain trees were constructed, including a reference genome for comparative purposes (supplementary figure 1), figure 2B shows the strain tree built for *Bacteroides caccae,* two distinct clusters corresponding to cyclists and weightlifters are revealed, indicating genetic differentiation between these groups.

Similar to *B. caccae*, *B. finegoldii* strains from weightlifters formed a tight cluster (Figure 2C). The short branch lengths in this cluster indicate a low genetic diversity, suggesting that the strains are highly similar. The analysis of *P. copri* presented a different pattern (Figure 2D), with no distinct clustering based on athletic groups. The strains from both cyclists and weightlifters were intermixed, indicating greater genetic diversity within this species.

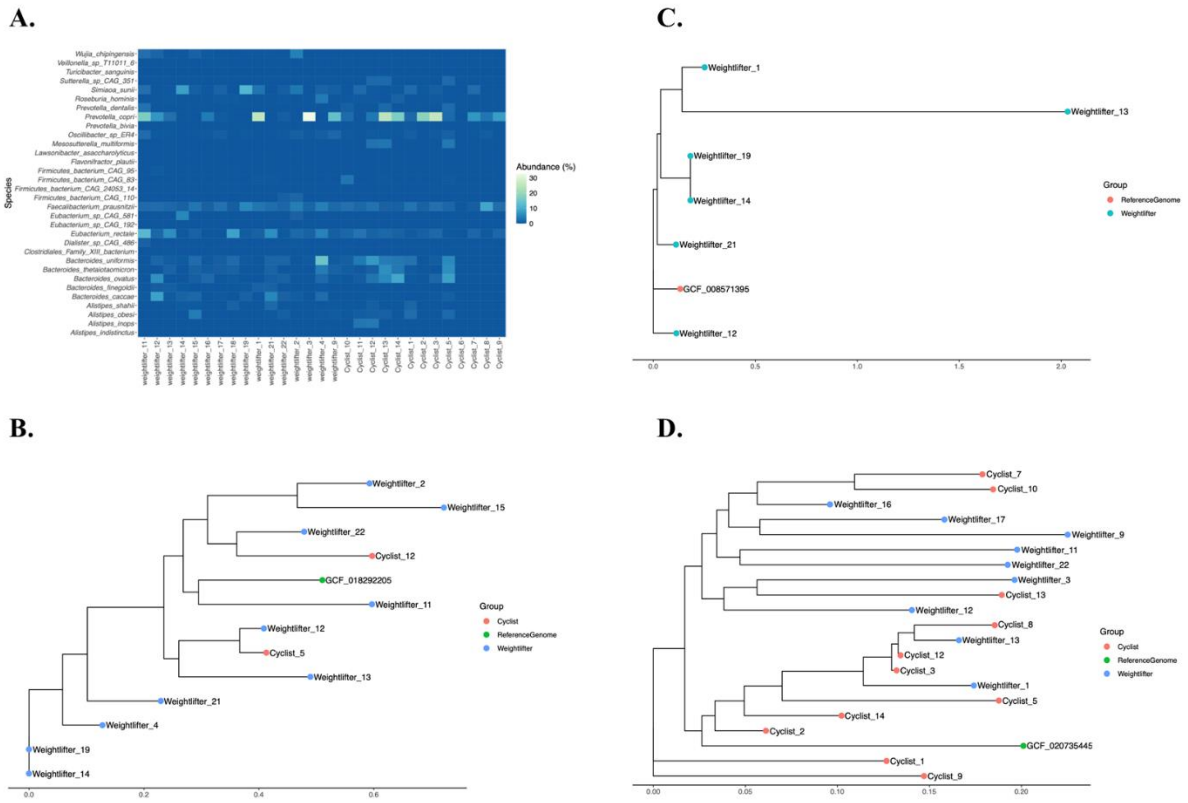


Fig 2. Comparative Analysis of Microbial Species Abundance and Strain Variation in Weightlifting and Cycling Athletes. 2A) Species level abundance: heatmap of filtered microbial species abundance in weightlifting athletes (WA) and cyclists (CA), with each cell representing the abundance of a species in a sample. Panels B-D present the results of StrainPhlAn4 analysis and alignment for the following species: **2B)** *Bacteroides caccae*, **2C)** *Bacteroides finegoldii*, and **2D)** *Prevotella copri*. Figures were created using the ggplot package from RStudio.

3.2 Functional Profiling of Fecal Samples from Professional Athletes

The raw metagenomic data obtained from fecal samples underwent processing using the HUMAnN4 pipeline [30]. This pipeline allows for the functional profiling of microbial communities by mapping sequence reads to reference databases, such as ChocoPhlAn, and subsequently annotating gene families and pathways. From the HUMAnN4 output, Enzyme Commission (EC), and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations were extracted for further analysis.

Figure 3A provides an overview of the functional pathways observed in the gut microbiota of professional road cyclists (CA) and weightlifters (WA), facilitating a comprehensive annotation and interpretation of functional pathways within their gut microbiota. The identified pathways in both groups encompass amino acid metabolism, carbohydrate metabolism, energy metabolism, glycan biosynthesis and metabolism, lipid metabolism, and metabolism of cofactors and vitamins.

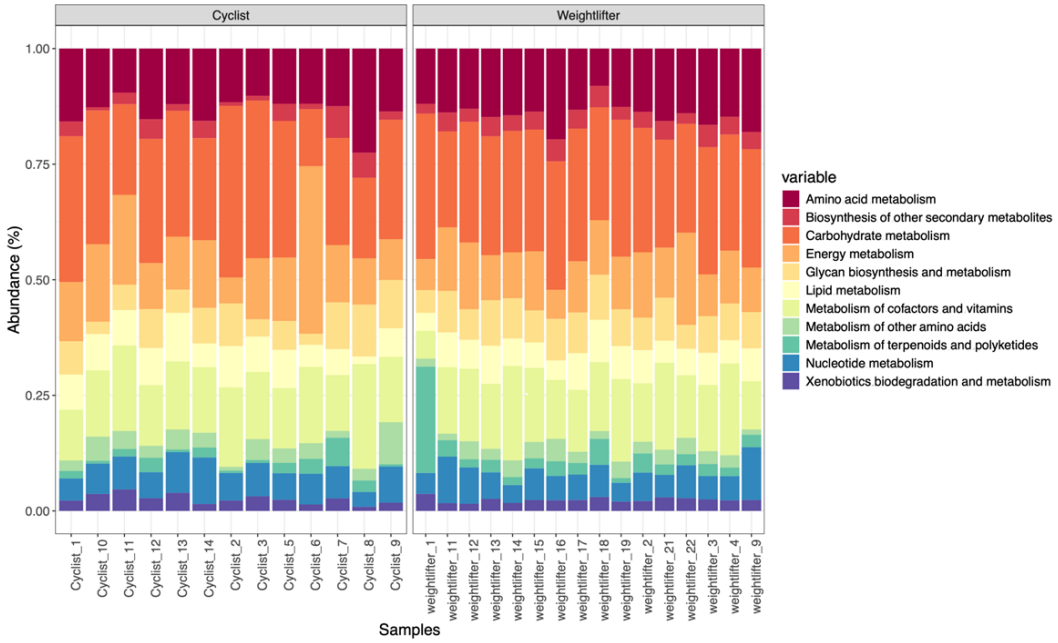
Analysis with MassLIN2 revealed distinct enzymatic activity profiles in the gut microbiota of weightlifters compared to road cyclists. Specifically, significant enrichment was observed in pathways such as L-arginine biosynthesis III (via N-acetyl-L-citrulline), fatty acid biosynthesis initiation (type II), streptorubin B biosynthesis, methylerythritol phosphate pathways I and II, GDP-mannose biosynthesis, pectin degradation I, glycine degradation, and 4-deoxy-L-threo-hex-4-enopyranuronate degradation. Notably, the bacteria *Bacteroides finegoldii* showed higher representation, indicating its significant role across the detected enzymatic reactions (Supplementary table 3).

To comprehensively explore these findings, Metacyc's omics system was utilized [37,45]. This platform facilitates the integration of metagenomic data in EC format, allowing for the visualization and recognition of significant pathways and enzymatic reactions across all datasets. The resulting integrated analysis provided a detailed metabolic pathway map highlighting distinctions between cyclists and weightlifters (Figures 3B-3D). Importantly, this analysis did not reveal any false discovery rates (FDR), underscoring the reliability of the identified metabolic pathways.

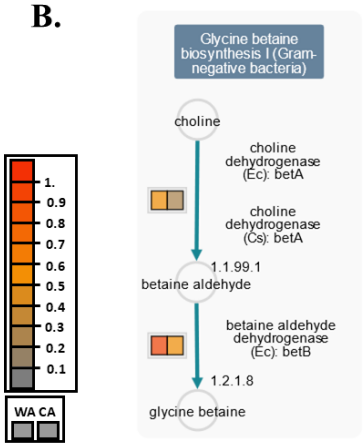
The analysis revealed that biosynthesis of amino acids appeared more prominently in weightlifters, whereas cyclists exhibited enhanced biosynthesis of fatty acids. Additionally, substrate degradation pathways, such as carbohydrates and amino acids, were more active in weightlifters compared to cyclists. A subtle difference was noted in the degradation of fatty acids in cyclists. Both groups showed fermentation to short-chain fatty acids, while cyclists exhibited pathways related to methanogenesis and weightlifters to the pentose phosphate pathway. Particularly, weightlifters gut microbiome contains representative enzymes that reflect the presence of pentose phosphate pathways (figure 3C).

Supplementary Table 3 list relevant enzymatic reactions, associated pathways, and EC code numbers obtained from HUMAnN4.0 analysis in Colombian athletes, (WA and CA).

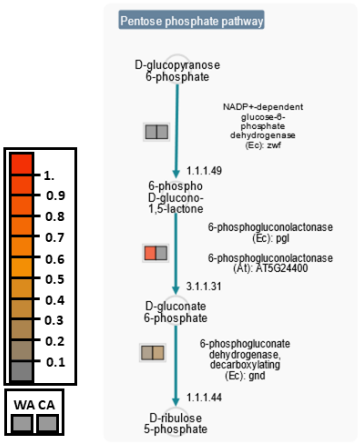
A.


Cyclist
Weightlifter
Abundance (%)
1.00
0.75
0.50
0.25
0.00
Cyclist_1
Cyclist_10
Cyclist_11
Cyclist_12
Cyclist_13
Cyclist_14
Cyclist_2
Cyclist_3
Cyclist_5
Cyclist_6
Cyclist_7
Cyclist_8
Cyclist_9
weightlifter_1
weightlifter_11
weightlifter_12
weightlifter_13
weightlifter_14
weightlifter_15
weightlifter_16
weightlifter_17
weightlifter_18
weightlifter_19
weightlifter_2
weightlifter_21
weightlifter_22
weightlifter_3
weightlifter_4
weightlifter_9
Samples
variable
Amino acid metabolism
Biosynthesis of other secondary metabolites
Carbohydrate metabolism
Energy metabolism
Glycan biosynthesis and metabolism
Lipid metabolism
Metabolism of cofactors and vitamins
Metabolism of other amino acids
Metabolism of terpenoids and polyketides
Nucleotide metabolism
Xenobiotics biodegradation and metabolism

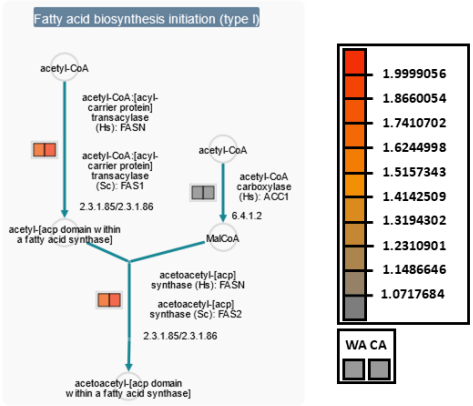
B.


Glycine betaine biosynthesis I (Gram-negative bacteria)
choline
choline dehydrogenase (Ec): betA
choline dehydrogenase (Cs): betA
1.1.99.1
betaine aldehyde
betaine aldehyde dehydrogenase (Ec): betB
1.2.1.8
glycine betaine
1.
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
WA CA

C.


Pentose phosphate pathway
D-glucopyranose 6-phosphate
NADP+-dependent glucose-6-phosphate dehydrogenase (Ec): zwf
1.1.1.49
6-phospho D-glucono-1,5-lactone
6-phosphogluconolactonase (Ec): pgl
6-phosphogluconolactonase (At): AT5G24400
3.1.1.31
D-gluconate 6-phosphate
6-phosphogluconate dehydrogenase, decarboxylating (Ec): gnd
1.1.1.44
D-ribulose 5-phosphate
1.
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
WA CA

D.


Fatty acid biosynthesis initiation (type I)
acetyl-CoA
acetyl-CoA:[acyl-carrier protein] transacylase (Hs): FASN
acetyl-CoA:[acyl-carrier protein] transacylase (Sc): FAS1
2.3.1.85/2.3.1.86
acetyl-[acp domain within a fatty acid synthase]
acetyl-CoA
acetyl-CoA carboxylase (Hs): ACC1
6.4.1.2
MalCoA
acetoacetyl-[acp] synthase (Hs): FASN
acetoacetyl-[acp] synthase (Sc): FAS2
2.3.1.85/2.3.1.86
acetoacetyl-[acp domain within a fatty acid synthase]
1.9999056
1.8660054
1.7410702
1.6244998
1.5157343
1.4142509
1.3194302
1.2310901
1.1486646
1.0717684
WA CA

427

Fig 3. Functional profiling of the gut microbiome of weightlifters and cyclist. 3A) Functional pathways: Overview of the functional pathways within the gut microbiota of professional road cyclists and weightlifters annotated by utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG), non-statistical differences were discovered. For figure 3B-3D, MetaCyc analysis was performed using the omics dashboard with Enzyme Commission (EC) codes to generate these pathways. The color scheme in these figures represents the relative abundance of EC in each group: weightlifters (WA) and cyclists (CA). Higher abundance in one group over the other is depicted through gradations of color intensity, from grey to red tones, convention color indicates major or less abundance of representative enzymes for each of the pathways selected. **Figure 3B)** Pathways related to **glycine biosynthesis**. **Figure 3C)** Representation of the **pentose phosphate pathway**. **Figure 3D) Fatty acid biosynthesis pathways.** Figure3A was created with ggplot package from RStudio, figures 3B – 3D were created with the omics dashboard from Metacyc server.

3.3 Metabolomic analysis of two different sport activities

To gain deeper insights into potential distinctions between athletes predominantly using glycolytic pathways (such as weightlifters) and those favoring oxidative pathways (like road cyclists), we conducted an integrative omics analysis. This comprehensive approach included the examination of fecal metabolome, as well as plasma metabolomic and lipidomic analyses.

3.3.1 Metabolomic Analysis of Fecal Samples

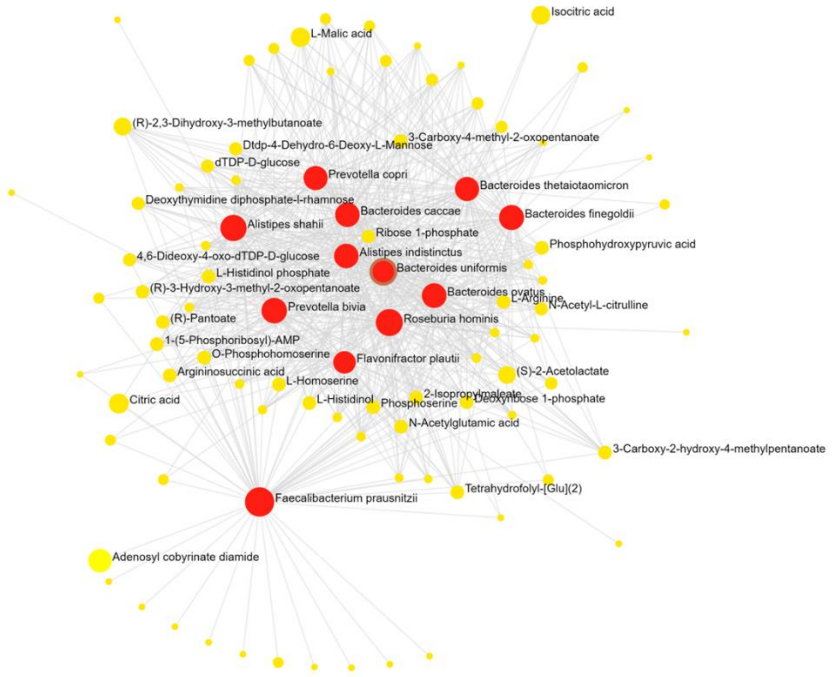
First, the metabolomic analysis of fecal samples from WA (n = 16) and CA (n = 13) was performed through the implementation of Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) platforms. The data underwent diverse procedures as detailed in the methods section and figure 1. Before the annotation process [40], platform stability was confirmed by performing quality control (QC) assessments, ensuring consistent performance across all platforms. The clustering of QC samples is shown in Supplementary Figure 2. Next, we obtained a set of metabolites listed in supplementary table 4. Statistical analysis was performed, nevertheless multivariate models did not reveal different profiles between groups, univariate analysis showcased diverse metabolites with significant abundances (p-value, FDR ≤

0.05). Figure 4A, showcase results from t-test analysis were metabolites like O-behenoyl carnitine, hydroxy-gamma-tocotrienol, docosadienoyl carnitine, beta-alanine betaine, arachidyl carnitine, and LPA 21:0, indicated statistically significant differences in their abundances between the two groups of athletic disciplines, with a higher abundance of fatty acyls in the weightlifter group. Next, we looked at those possible metabolites produced by the gut microbiota, reported metabolites such as: glycolate, Hydroxy butyric acid , nicotinate, beta-alanine betaine, ethanolamine, L-valine, dopamine, histidinal, hyodeoxycholate, among others were classified as theoretically produced by the gut microbiota.

After identifying these metabolites, a knowledge-driven omics analysis was performed with the species identified through MetaPhlAn4 (Figure 2A) and the subset of metabolites produced by the gut microbiota). This integrative approach utilized OmicsNet 2.0 [38] to explore potential interactions between microbial species and metabolites (Figure 4)[42].

The resulting network highlighted key microbial species and their associated metabolites (figure 4), providing insights into how specific gut microbiota might contribute to the metabolic profiles observed in weightlifters and road cyclists. This approach facilitated a comprehensive understanding of the potential biological mechanisms underlying the differences in metabolite production and metabolic pathways between the two groups of athletes.

Using OmicsNet, we mapped interactions and identified key nodes in the network for potential further investigation. The integrative analysis emphasizes the value of considering both microbial and metabolomic data to unravel the complex relationships between physical activity, gut microbiota, and host metabolism. In this model, the yellow dots represent metabolites hypothetically produced by the gut microbiota and identified through fecal metabolomic analysis, while the red dots indicate microbial species identified using the MetaPhlAn4.0 pipeline (Figure 2A). The OmicsNet analysis revealed enriched metabolic pathways, which are graphically represented by the nodes in Figure 4. The pathways showing significant correlations between species and metabolites include phenylalanine, tyrosine, and tryptophan biosynthesis; arginine biosynthesis; valine, leucine, and isoleucine biosynthesis; histidine metabolism; folate biosynthesis; and vitamin B6 metabolism, as detailed in Table 1.



485

486 **Fig 4. Fecal metabolomics and integrative analysis. Network Analysis of Microbial Species**
487 **and Metabolites:** This network diagram illustrates the connections between key microbial species
488 and their associated metabolites. Red dots represent microbial species, yellow dots denote
489 metabolites, and the lines indicate significant correlations between them [41]. The prediction of
490 metabolic potential for different microbial taxa was conducted using the OmicsNet web server
491 [41], which employs logistic regression models based on high-quality genome-scale metabolic
492 models (GEMs). This tool enables further enrichment of the network by integrating protein-
493 metabolite interactions to identify potential enzymes as shown in table 1.

494

495 **Table 1: Pathway enrichment analysis obtain from metagenomic data from gut microbiome**
496 **of Colombian weightlifters and road cyclists.**

Pathway	FDR	P.Value
Phenylalanine, tyrosine and tryptophan biosynthesis	4.53E-07	3.31E-09
Arginine biosynthesis	4.53E-07	3.73E-09
Valine, leucine and isoleucine biosynthesis	4.53E-07	3.73E-09
Histidine metabolism	7.04E-05	7.71E-07

Folate biosynthesis	0.000216	2.96E-06
Citrate cycle (TCA cycle)	0.0387	0.00138
Biosynthesis of secondary metabolites - other antibiotics	0.0523	0.00201
Glycine, serine and threonine metabolism	0.057	0.00234
Glucagon signaling pathway	0.0684	0.003
Pentose phosphate pathway	0.122	0.00702
Glycerophospholipid metabolism	0.29	0.0207
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	0.383	0.0336
Vitamin B6 metabolism	0.424	0.0395

FDR: False discovered

3.4 Plasma metabolome and lipidome from Colombian athletes with different energy demand

With the aim of identifying metabolic differences within the blood matrix of athletes, with a specific focus on reflecting potential variations between two distinct groups: athletes predominantly utilizing glycolytic pathways (Weightlifters) and those primarily relying on oxidative metabolism (Cyclists) a metabolomic and lipidomic approaches were employed.

Through the utilization of metabolomic techniques, this assay aimed to provide a comprehensive profile of the small molecule metabolites found in athletes' plasma blood samples. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) were employed for the analysis of plasma metabolome from WA (n = 16) and CA (n = 13).Following the annotation process, approximately 120 compounds were identifiedand subsequently, a reduction analysis was performed using linear modeling [46]. The resultant metabolites from this analysis are highlighted in supplementary table 5 and visualized in Figures 5B.

Different classes of metabolites were detected, including carboxylic acids, carbohydrates, dicarboxylic acids, fatty acids, fatty acyls, indoles, organic carbonic acids, organonitrogen compounds, sphingolipids, steroids, sterols, and tricarboxylic acids, among others (Supplementary Table 5). Among the identified compounds, glycerophosphocholines and glycerophospholipids

were the most abundant. Significant differences were observed in families such as glycerophosphoethanolamines, medium-chain fatty acids, monoradylglycerols, steryl esters, and dicarboxylic acids, with greater abundance in plasma samples from the WA group compared to the CA group ($p \leq 0.05$; FDR ≤ 0.05). In contrast, metabolites with higher abundance in the CA group were predominantly from the subclass triradylglycerols.

In addition to multivariate analysis (Figure 5A), univariate analysis was conducted to identify significant differences between groups (Figure 5B).

The multivariate models (MVA) demonstrated successful group separation, as evidenced by the distinct clustering in the score plots. Model quality was evaluated using R^2 and Q^2 values, confirming strong predictive power and explained variance. Specifically, the model accounted for 45.4% of the cumulative variation in X (metabolite data) and 98.9% in Y (group), with a high cumulative predictive ability of 76.6% and a significant p-value of 2.0283e-05. These results emphasize the effectiveness of the OPLS-DA model in distinguishing between different metabolic profiles, highlighting the importance of the observed differences. Additionally, univariate analysis (UVA) was performed to further explore the significance of individual metabolites, complementing the findings from the MVA.

To further identify significant features between groups, fold change and t-tests were applied (supplementary table 5). Figure 5B displays major differences foundin the weightlifter group (WA), with increased levels of monoacylglycerol (MG 18:0), triglyceride (TG 31:0), medium chain fatty acids (FA 18:2), lysophosphatidylcholine (LPC 18:3), caprylic acid, and diacylglycerol (DG 27:0).

To provide a comprehensive overview of the top 25 features identified through the plasma metabolomic analysis, a heatmap was constructed. The selection was based on significant changes observed, with a p-value and FDR threshold set to ≤ 0.05 using MetaboAnalyst 6.0 [43] to prioritize these compounds. This approach allowed us to focus on the most relevant features, which are visually represented in Supplementary Figure 3, highlighting key metabolic signatures related to WA and CA groups.

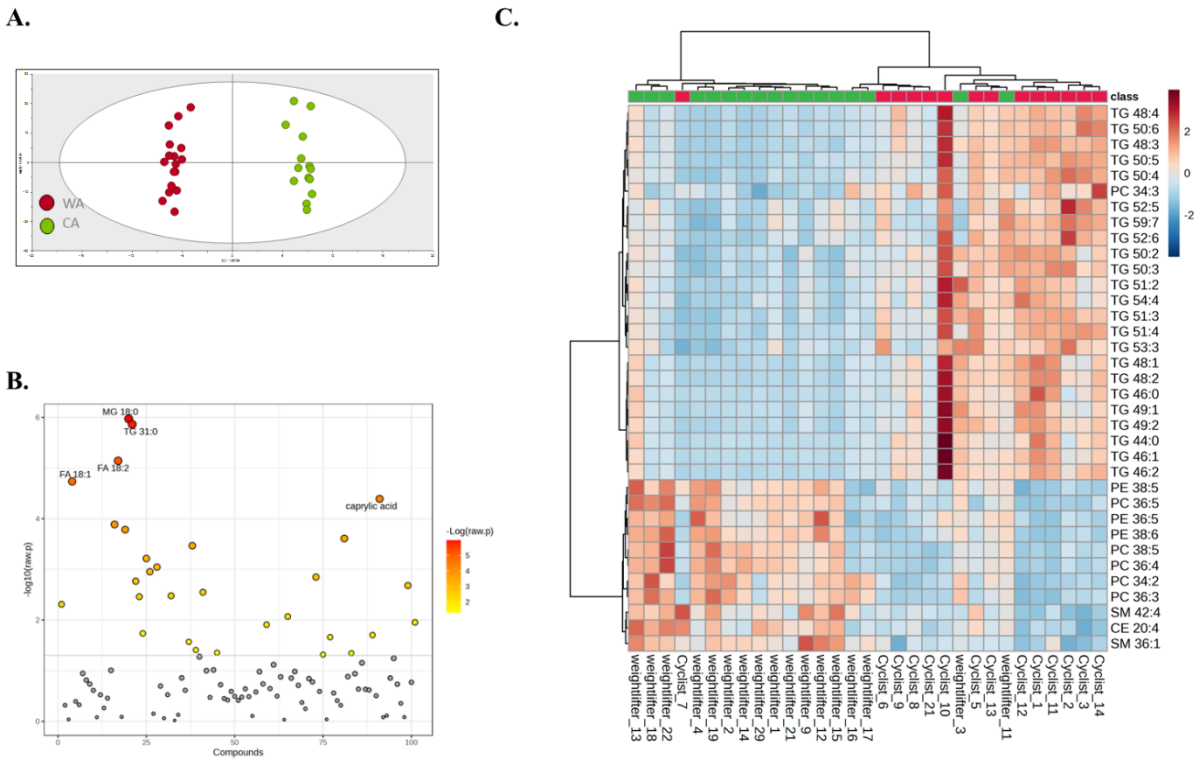


Fig 5. Plasma metabolome and lipidome from Colombian athletes with different energy demand. 5A) Orthogonal partial least squares discriminant analysis (OPLS-DA): reveals significant separation between the sample groups, with notable performance metrics. **5B) T-test analysis** was conducted to visualize the differences in metabolite abundance between the two types of sports (WA - weightlifters and CA - cyclists). **5C) Hierarchical Clustering Heatmap from lipidomic analysis:** This analysis provides a comprehensive overview of the lipidomic species identify across the blood samples of WA and CA groups. Notably, triglycerides have a strong abundance profile in cyclists, meanwhile other types of lipids like sterols and phosphocholines are more abundant in weightlifters. Graphics and comprehensive analysis were performed with Metaboanalyst 6.0 [43].

3.5 Lipidomic analysis

Multivariate and univariate statistical analyses, such as principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and t-tests, were utilized to identify differential lipid species and elucidate metabolic patterns associated with experimental conditions.

The Orthogonal partial least squares discriminant analysis points a robust separation between sample groups based on their plasma lipidomic profiles. This model exhibits high performance metrics, $R^2X(cum)$: 0.626 $R^2Y(cum)$: 0.984 $Q^2(cum)$: 0.808 and a significant p-value (3.14953e-05) (Supplementary figure 4A).

Plasma lipidomics analysis revealed distinct lipid profiles between experimental groups, with significant differences observed in the abundance of specific lipid species. The lipid species demonstrating the most notable disparities in abundance were identified, namely triglycerides (TG) and phosphatidylcholine (PC).

As shown in figure 5C and supplementary figure 4B, triglycerides (TG) were significantly elevated in cyclists compared to weightlifters. This observation suggests a heightened reliance on lipid metabolism and energy utilization during endurance exercise. TGs serve as an essential energy source during prolonged aerobic activities, such as cycling, where sustained effort demands efficient lipid oxidation for ATP production. The specific TG species identified, including TG54:6, TG52:4, and TG54:3, may reflect adaptations to endurance training, facilitating enhanced lipid mobilization and utilization to sustain energy production over extended periods. Elevated levels of TGs with shorter carbon chains, such as TG50:2 and TG50:1, may indicate increased lipolysis and fatty acid turnover, supporting energy production during endurance exercise bouts. An enrichment analysis performed with LION/web [47,48], highlight the upregulated pathways that conduct to lipid droplet formation, lipid storage, glycolipids synthesis, among other (supplementary table 6, supplementary figure 4B-4C).

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) species are notably enriched in weightlifters compared to cyclists. PC and PE are essential components of cell membranes and play critical roles in membrane integrity, signaling, and lipid metabolism (Supplementary figure 4C).

4 Discussion

Our study aimed to explore the intricate metabolic patterns associated with weightlifting and road cycling. Using various omics techniques (Figure 1), we investigated potential pathways linking athletes' gut microbiomes to their systemic metabolism. These research findings contribute to the growing body of evidence suggesting that the gut microbiota of athletes may experience metabolic

adjustments to support energy metabolism and bolster physiological resilience during physical activity [10,19,44,48–53]. However, further research is needed to fully understand these mechanisms [20]. Future studies should explore how these microbial changes could be leveraged to optimize training protocols or nutritional interventions for different types of athletes, potentially leading to more personalized approaches in sports science. This could ultimately contribute to the development of individualized training and nutritional strategies that enhance athletic performance.

Despite not reporting robust differences between the taxonomic and functional microbiota of both groups (WA - CA) as seen in multivariate and univariate analyses (see Supplementary Table 2), our MetaPhlAn4.0-incorporated analysis provides a valuable glimpse into the gut microbiota of Colombian athletes as showed in Figure 2A and Supplementary Table 1, here we detail the most abundant species identified in both athlete groups, including genera such as *Bacteroides, Eubacterium, Prevotella, Alistipes,* and *Faecalibacterium.* These genera are known for their roles in various metabolic processes [54], and their abundance in the microbiota could reflect adaptations to the specific physical demands of weightlifting and cycling [26] [50,55]. These findings are in concordance with other related research, where gut microbiota members are recognized for their metabolic benefits to the host [9,56–59]. Moreover, since we captured the microbiota just before a competition, future research could benefit from analyzing multiple samples taken during different phases, such as pre- and post-competition or during preparation periods [19,20,25,60], to further understand these adaptations.

The relevance of *Bacteroides* species in the gut microbiota of athletes is underscored by their ability to break down a wide range of dietary polysaccharides through carbohydrate-active enzymes (CAZymes) [61], producing metabolites like acetate, propionate, and succinate, which play crucial roles in maintaining gut mucosal integrity and immune defenses [62]. Additionally, *Bacteroides* species exhibit diversity at the species and strain level in their production of short-chain fatty acids, influenced by factors such as diet and physical activity [50,55,63]. This metabolic flexibility may contribute to their prevalence in athletes, where different physical activities demand specific metabolic adaptations [55]. Moreover, *Bacteroides* are involved in the modification of bile acids, impacting host metabolism and immune function, which could further explain their

significant presence in athletes [53]. Further analysis using StrainPhlAn4 identified two specific *Bacteroides* strains, *Bacteroides caccae* (Figure 2B) and *Bacteroides finegoldii* (Figure 2C), which were associated with weightlifters and road cyclists. *B. caccae* has been previously linked to sports with high dynamic and static components, such as rowing [2], suggesting its role in supporting the physical demands of these activities.

Although *B. finegoldii* has not been directly associated with sports, it has been identified in highly active military subjects, hinting at its potential relevance to physically demanding lifestyles [55]. Moreover, we identified *Bacteroides thetaiotaomicron* (Supplementary table 1, figure 2A), that is known for its ability to break down complex carbohydrates, enhancing nutrient absorption and energy availability, which is particularly beneficial for athletes who require efficient energy metabolism during training and competition. Research also indicates that *B. thetaiotaomicron* is present in the gut microbiota of esports players [64], suggesting its potential role in maintaining gut health in competitive environments. Its presence may contribute to the overall metabolic health of athletes, supporting their performance through improved digestion and nutrient utilization. Similarly, *Bacteroides uniformis* plays a crucial role in carbohydrate fermentation and is associated with the production of SCFAs, which are beneficial for gut health and may influence inflammation and recovery in athletes [52]. Studies have shown that *B. uniformis* is often found in the gut microbiota of physically active individuals, including athletes, where its presence is linked to a balanced gut microbiome [53]. Although our analysis identified specific *Bacteroides* species and performed strain analysis through StrainPhlAn4, these results require further confirmation. Future studies should aim to correlate these findings with other potential factors, such as diet and environmental conditions, to gain a more comprehensive understanding of the role of these microbes in athletic performance.

Similarly to the *Bacteroides* genus, we identified diverse *Prevotella* species in our analysis, including *Prevotella copri*, *Prevotella stercorea*, *Prevotella bivia*, and *Prevotella dentalis*. Although no statistical differences were detectable between the athlete groups, these results align with previous reports that have established *Prevotella* as a prominent member of the gut microbiota in cyclists, particularly in north America [65]. *Prevotella* species, especially *P. copri*, are known for their involvement in the metabolism of complex carbohydrates. As seen in Figure 2A,

Prevotella copri stands out for its higher abundance across samples, emphasizing its potential role in the gut microbiota of athletes. They play a crucial role in fermenting dietary fibers into short-SCFAs, which can be utilized as an energy source during prolonged physical activity [66]. This metabolic capability is particularly advantageous for endurance athletes, such as cyclists, who rely on sustained energy release during extended periods of exercise [44]. Unfortunately, we were unable to correlate these findings with dietary data due to its absence in this study. However, future research could aim to include detailed dietary data to distinguish the types of carbohydrates and fibers consumed by athletes, potentially enhancing our understanding of how specific diets influence gut microbiota composition and function in sports performance.

Through HUMAnN4 analysis, we identified numerous carbohydrate degradation enzymes from gut microbiota family members. Although no statistical differences were observed, the MetaCyc omics dashboard revealed the presence of multiple pathways involved in polysaccharide degradation and carboxylic acid degradation [45]. Fecal metabolomic assays further allowed us to identify key metabolites such as oxalate, propanoate, succinate, glycolate, among others (figure 4 and supplementary table 4), reflecting the metabolic activities of the gut microbiota and their potential contributions to the athletes' performance. To further elucidate these metabolic processes and gain a more comprehensive understanding, incorporating additional omic approaches, such as RNA sequencing [67] and single-cell metabolomics [68], could be invaluable. These advanced techniques would allow to explore gene expression patterns and metabolic activity at a more specific level, providing deeper insights into the functional roles of gut microbiota in athletic performance and how these may vary between individuals or under different environmental conditions.

Our integrative omics analysis further revealed that the enrichment pathways visualized from the integration of metagenomic, and metabolic data demonstrated a significant relationship ($p < 0.005$) with the biosynthesis of phenylalanine, tyrosine, and tryptophan, as well as the biosynthesis of arginine, valine, leucine, and isoleucine (Table 1). These pathways are critical for protein synthesis and metabolic functions that support athletic performance. The functional analysis of enzymes also revealed a significant presence of enzymes encoding for amino acid synthesis, which complements our fecal and plasma metabolome analysis that identified the presence of L-valine, a key BCAA

(Figure 4B). Although these findings suggest that *Prevotella* species may contribute to the enhanced metabolic capacity of athletes, supporting both energy production and muscle recovery, we did not perform any correlation analysis in this study. As a result, we cannot confirm that *Prevotella* or any other species are directly related to the findings of amino acids or BCAAs in the metabolome analysis. However, future research could explore these potential connections, offering a deeper understanding of the role of gut microbiota in athletic performance.

The integrative analysis performed in this study has provided significant insights into the metabolic functions of key microbial genera, as illustrated in Figure 4 and Table 1. These microbial activities are essential for energy production, muscle recovery, and overall athletic performance (Figure 3B-3D). Building on these findings, our lipidomic analysis further elucidated the metabolic landscape in athletes (Figure 5C). The examination of metagenome and metabolome data revealed a prominent presence of lipids and lipid-like substances across both groups of athletes (see Figure 4 and Figure 5). Specifically, our analysis of fecal metabolites identified significant quantities of various lipid classes, including fatty acyl carnitines, amino acid derivatives, steroids, phosphatidylcholines (PC), and phosphatidylethanolamines (PE), as exhibited in supplementary table 4 and table 2. These lipid metabolites are critical for energy metabolism, cellular signaling, and membrane integrity, all of which are vital for maintaining high levels of physical performance [22,27,69,70]. Enrichment analysis of lipidome results revealed upregulated pathways linked to lipid droplet formation, lipid storage, and glycolipid synthesis (Supplementary Table 6, Supplementary Figure 4B-4C), emphasizing the importance of these processes in enhancing athletic performance.

To further explore these interactions, Figure 6 presents a schematic representation that hypothesizes potential links between gut microbiome metabolism and host metabolome/lipidome. This figure offers a conceptual framework for understanding the interplay between gut microbial activity and metabolite production. However, it is important to note that these connections are hypothetical and require further investigation to fully map out and validate the comprehensive metabolic relationships involved. Previous studies have shown that athletes exhibit higher levels of fecal metabolites such as SCFAs like butyrate, acetate, and propionate [64,71], ammonia [72], and amino acids and their derivatives. Although we did not describe SCFAs in detail in our current

study, we identified 3-hydroxybutanoate (see supplementary table 4 and figure 4B) in fecal metabolome and detect related pathways in MetaCyc´s dashboard [45]. This metabolite, found in both fecal and plasma samples from weightlifters and cyclists, suggests a significant role in the metabolic processes of these athletes, particularly concerning fatty acid and lipid biosynthesis/degradation and cofactor synthesis. Sub-pathways related to phosphatidylcholine synthesis, phosphatidylcholine resynthesis via glycerophosphocholine, and plasmalogens were also identified (Figure 6).

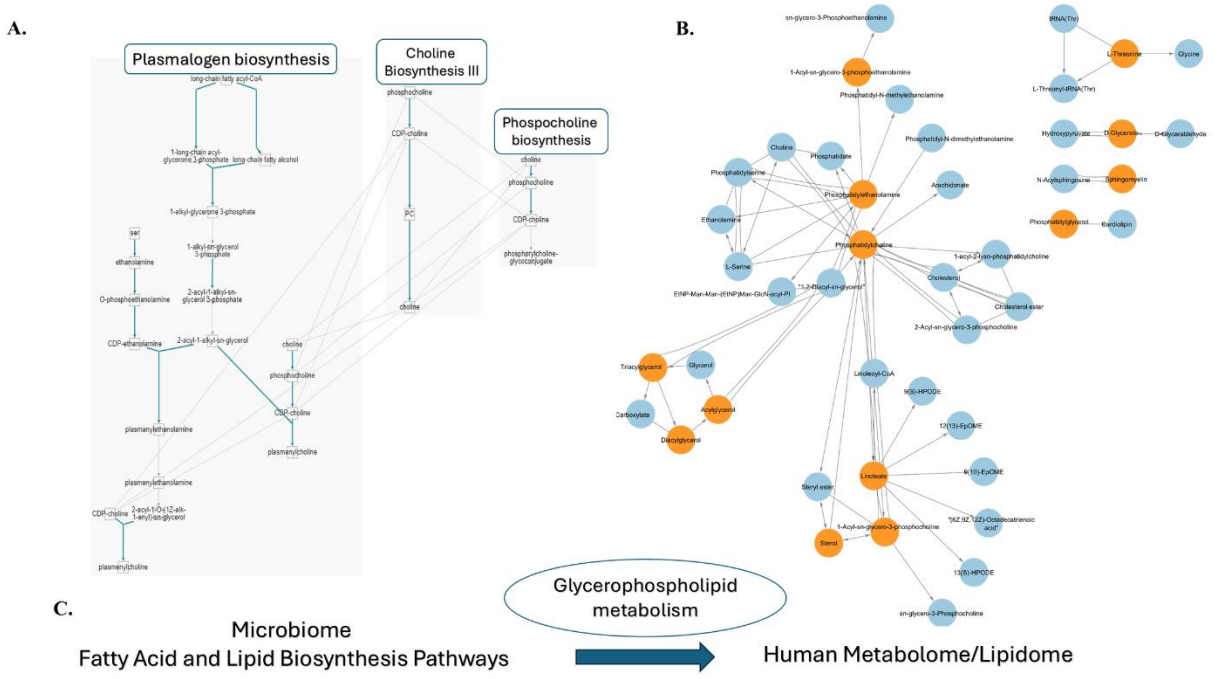


Fig 6. Hypothetical Framework Illustrating Potential Interactions Between Gut Microbiome Metabolic Reactions and Key Metabolite Classes and Pathways. This figure presents a schematic representation illustrating potential links between gut microbiome reactions identified through metagenomics analysis and key metabolite classes along with their associated pathways. Figure **6A)** Shows specific biosynthesis pathways, including **Plasmalogen biosynthesis**, **Choline Biosynthesis III**, and **Phosphocholine biosynthesis**. Pathways were built with the representative enzymes from metagenomic data [45]. Graphic 6A was created through the pathways analysis tool from Metacyc's server. **Figure 6B)** depicts **a network of metabolites interconnected by their potential associations**, with glycerophospholipid metabolism highlighted as a central theme. The network B was constructed using Cytoscape 3.0 software [74]. Significant metabolites and lipids

identified from the metabolomic and lipidomic data are represented by orange circles, while the enriched pathways are shown in blue circles. **Figure 6C)** Illustrates the broader context, showing the interaction between **microbiome fatty acid and lipid biosynthesis pathways** and the human **metabolome/lipidome**. The connections depicted in this figure are hypothetical and serve as a conceptual framework for understanding the interplay between gut microbial activity and human metabolite/lipid production.

Methodological differences, such as the use of untargeted versus targeted metabolomic analysis [73], the inclusion of different sports [2], and the assessment of acute versus chronic effects [72], as well as the inclusion of both professional and non-professional athletes, may influence the comparability of our results with previous findings. Despite these variations, our lipidomic analysis provides complementary evidence to the microbial and metabolic data, offering a more integrated perspective on how gut microbiota and their metabolic byproducts contribute to the physiological adaptations observed in elite athletes. Future research should continue to explore these relationships to further elucidate the mechanisms underlying athletic performance and to confirm possible connections between host and microbiota through lipid like molecules.

Our study hypothesized that weightlifting and cycling, which involve different energy systems, would be associated with distinct gut microbiome compositions and functional profiles. However, our metagenomic and fecal metabolome analyses did not reveal clear differences between these sports. Despite this, our plasma metabolome and lipidome analyses yielded significant findings. The multivariate analysis of plasma metabolomic and lipidomic data indicated meaningful differences that were not apparent in the metagenomic and fecal analyses.

The higher abundance of PCO-34:2, PC18:0, PEO-36:5, and PEO-38:5 in weightlifters suggests distinct lipid metabolic adaptations associated with resistance training and muscle adaptation, these lipid species may contribute to membrane remodeling processes and cellular adaptations to mechanical stress and metabolic demands characteristic of weightlifting activities. Furthermore, the presence of specific PC and PE species, such as PCO-36:5 and PEO-38:6, may reflect unique lipidomic signatures associated with muscle tissue remodeling and repair processes following intense resistance exercise.

This discrepancy highlights the importance of using a multi-omic approach to gain a comprehensive understanding of the metabolic adaptations associated with different types of physical activity. Our results suggest that while the gut microbiome may not vary significantly between weightlifters and cyclists in terms of composition or function, the systemic metabolic responses observed through plasma and lipidomic analyses provide valuable insights into how these athletes adapt to their specific training regimens.

Despite the insights provided by this study, various limitations must be acknowledged. The relatively small sample size, consisting of elite weightlifters and cyclists, limits the generalizability of our findings to other sports disciplines and broader athlete populations. Moreover, focusing exclusively on professional athletes may not fully represent the microbial and metabolic profiles of amateur or recreational athletes. Our inability to collect detailed dietary data from participants further restricts our capacity to correlate specific dietary patterns with observed microbial and metabolomic profiles. Comprehensive dietary assessments are essential for understanding how different types of carbohydrates, proteins, fats and fibers impact gut microbiota and metabolic outcomes. Although potential links between gut microbiota, metabolomic data, and lipidomic profiles were identified, direct correlation analyses with specific measures of physical performance were not performed. Future research should explore these relationships more explicitly to validate the role of microbial and metabolic factors in athletic performance. Lastly, methodological variability, including differences between untargeted and targeted metabolomic analyses, may affect the comparability of our results with those from other studies. Standardizing methodologies across research will enhance the robustness and reproducibility of findings in this field.

In conclusion, this study explores the intricate relationship between gut microbiota and athletic performance using diverse omics approaches. Key microbial genera like *Bacteroides* and *Prevotella* were found to be integral to metabolic functions essential for elite athletes, influencing energy metabolism, muscle recovery, and performance. The incorporation of lipidomic data enhanced our understanding of gut microbiota's role in lipid metabolism and cellular functions crucial for athletes. Although not all variables were addressed, this work provides a foundational framework for future research on how gut microbiota and metabolic profiles affect sports performance. The findings emphasize the complex interplay between gut microbiota, metabolic

pathways, and athletic outcomes, advancing our understanding of microbiome changes associated with different training regimens.

Ethics declarations

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Author Contributions

JDR, MC, VA designed the study; VA, DP, LV processed the samples and analyzed the data. VA and JDR drafted the manuscript. All authors approved the final version of the Manuscript.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Data Availability

Extracted data are available from the corresponding author upon reasonable request.

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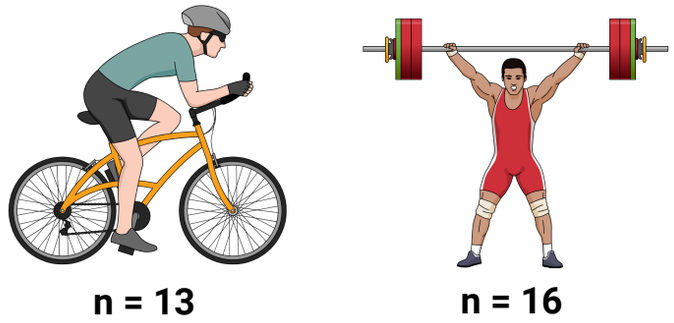

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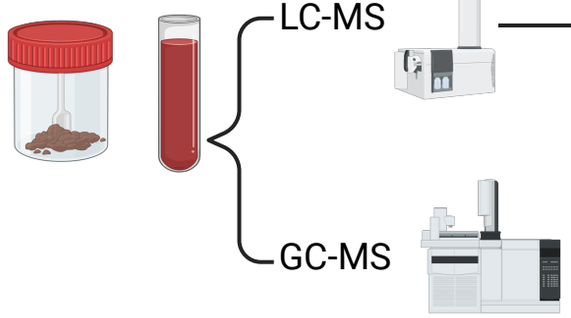
MetaPhlAn4

HUMAnN4

Metabolomics



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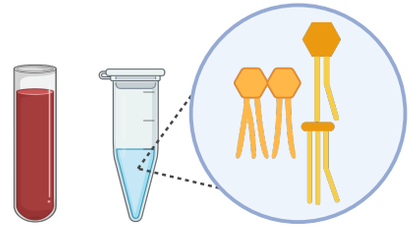


b)

Raw seqs

Taxonomic profiling

Functional profiling

Features

GP

AA

OC

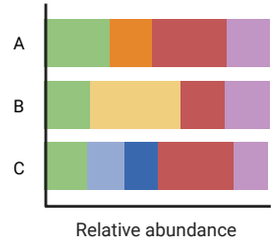
Features

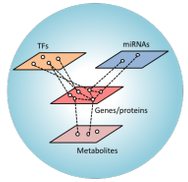
TG

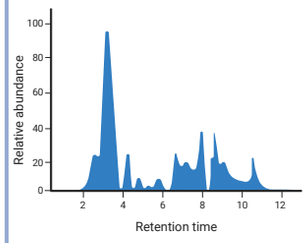
GPC

ST

c)







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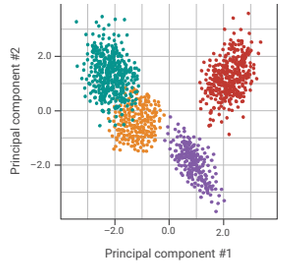

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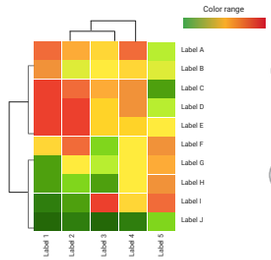

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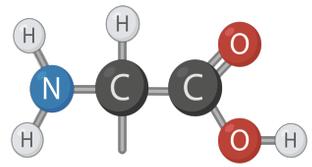

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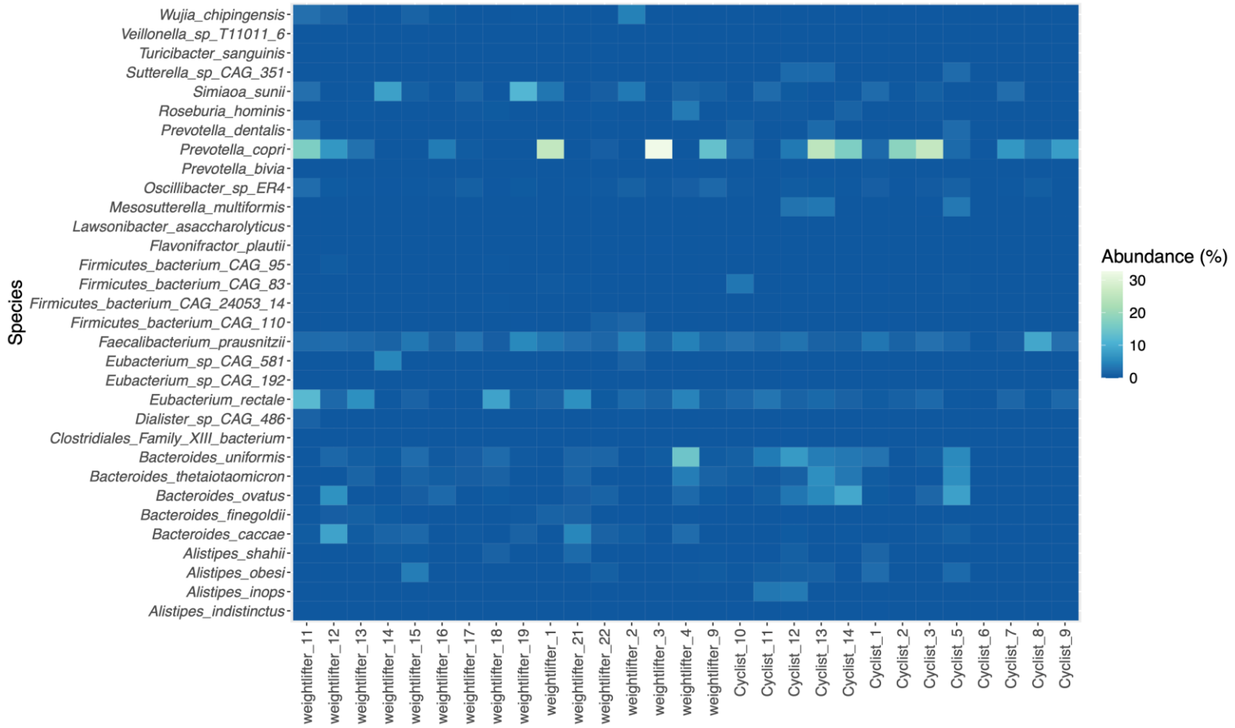

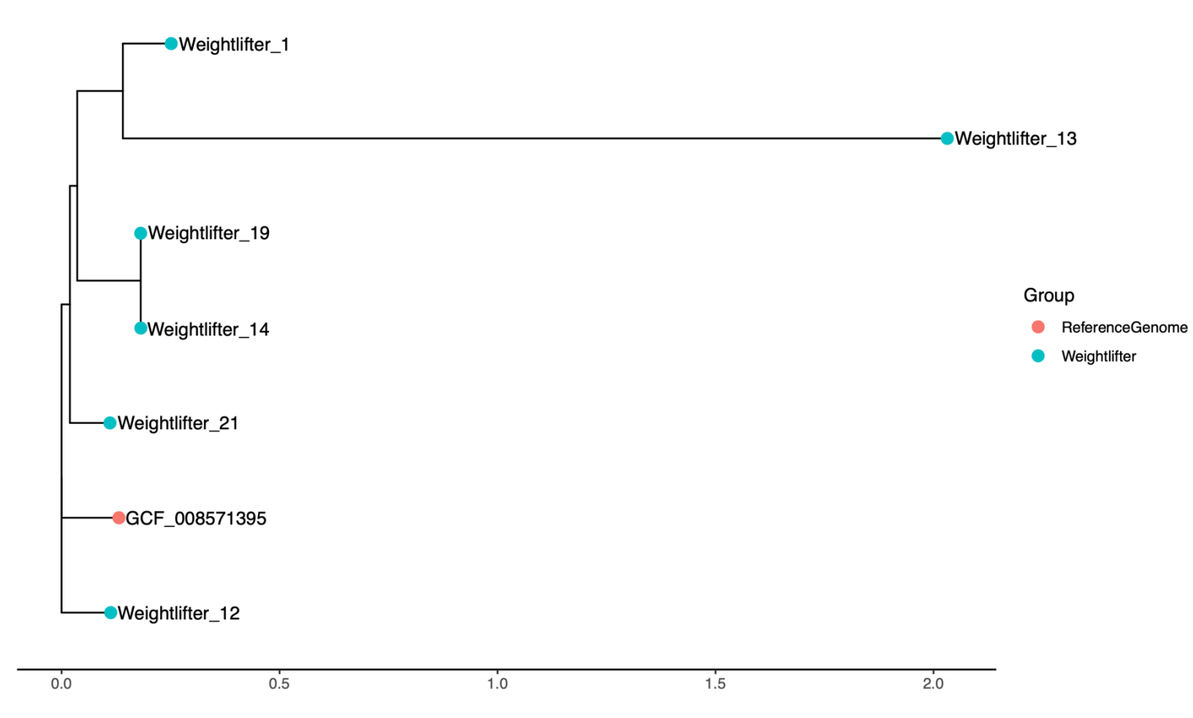

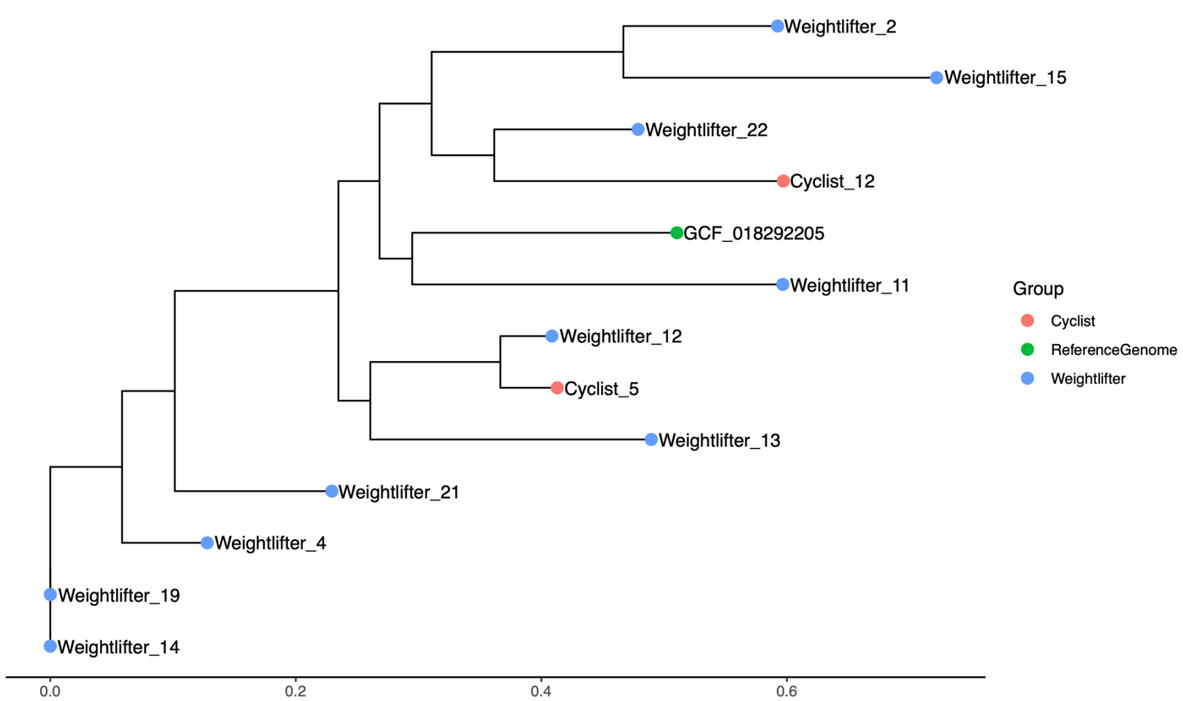

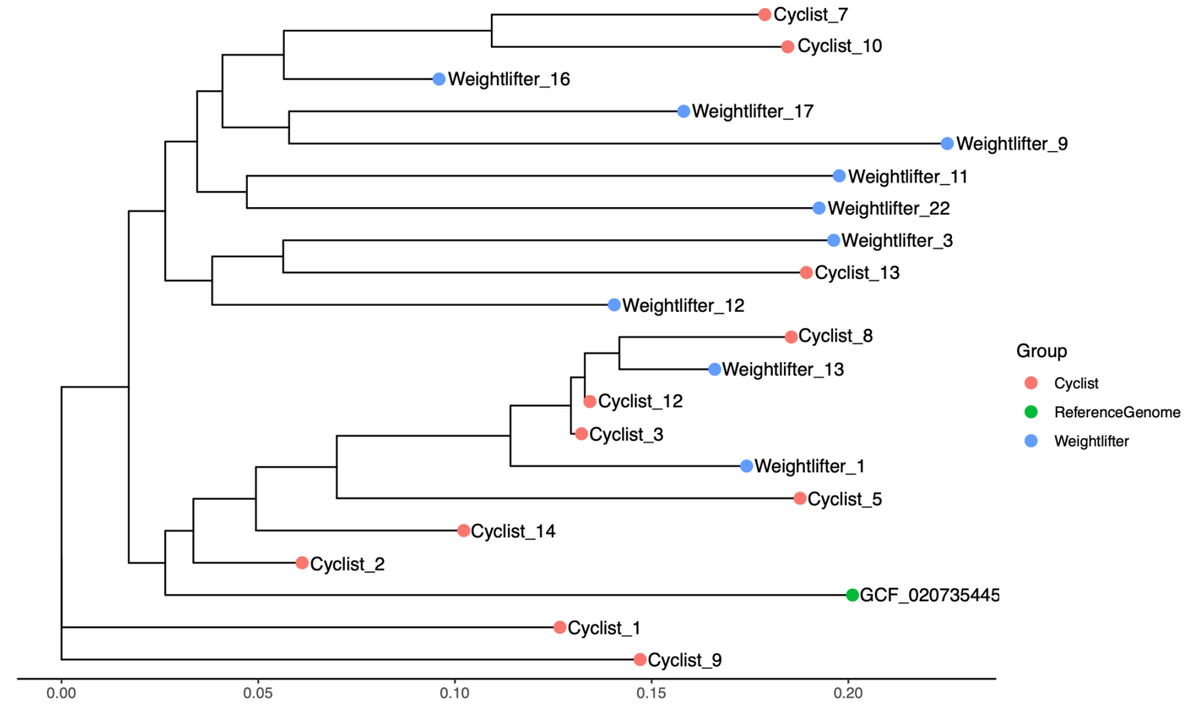

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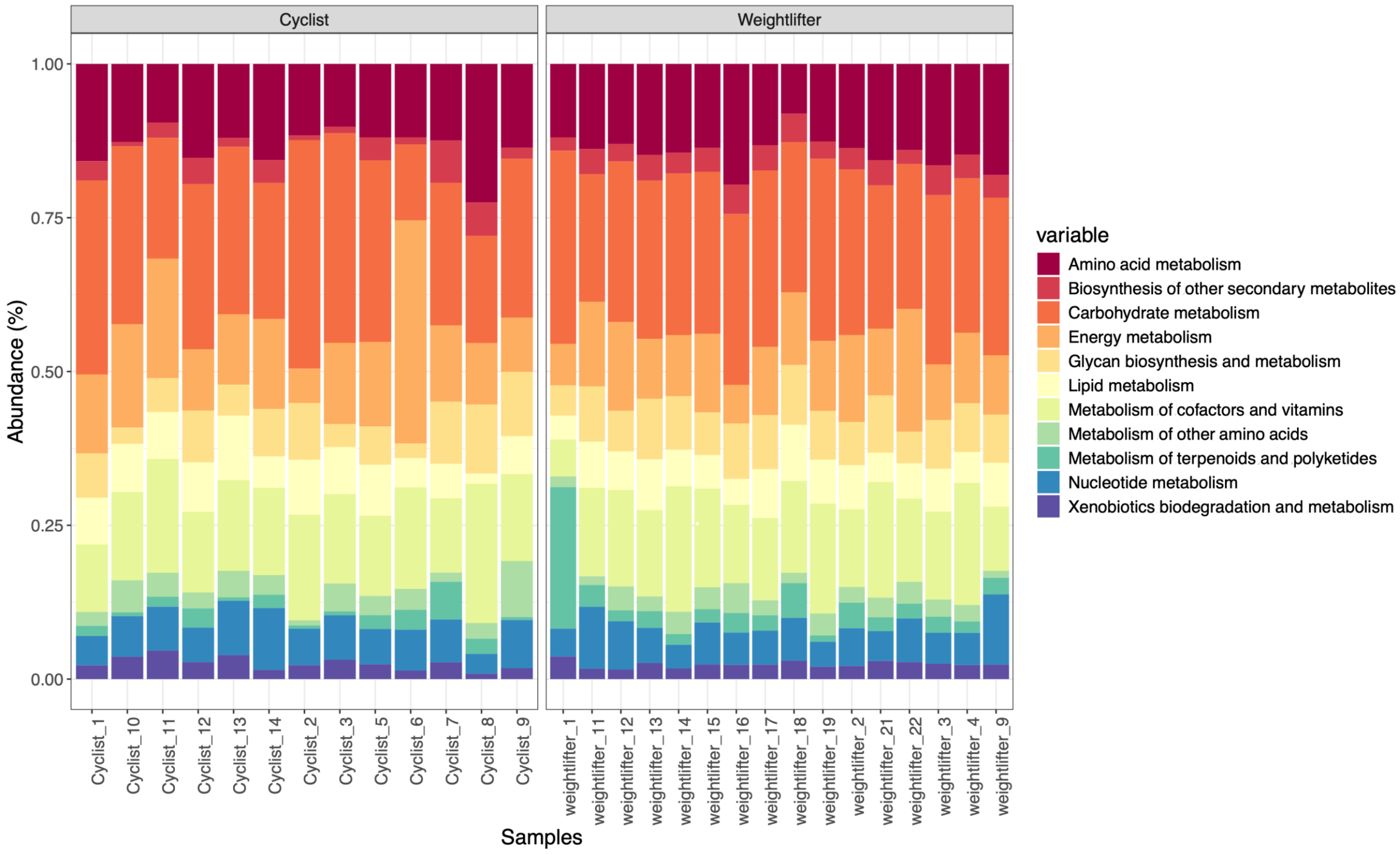

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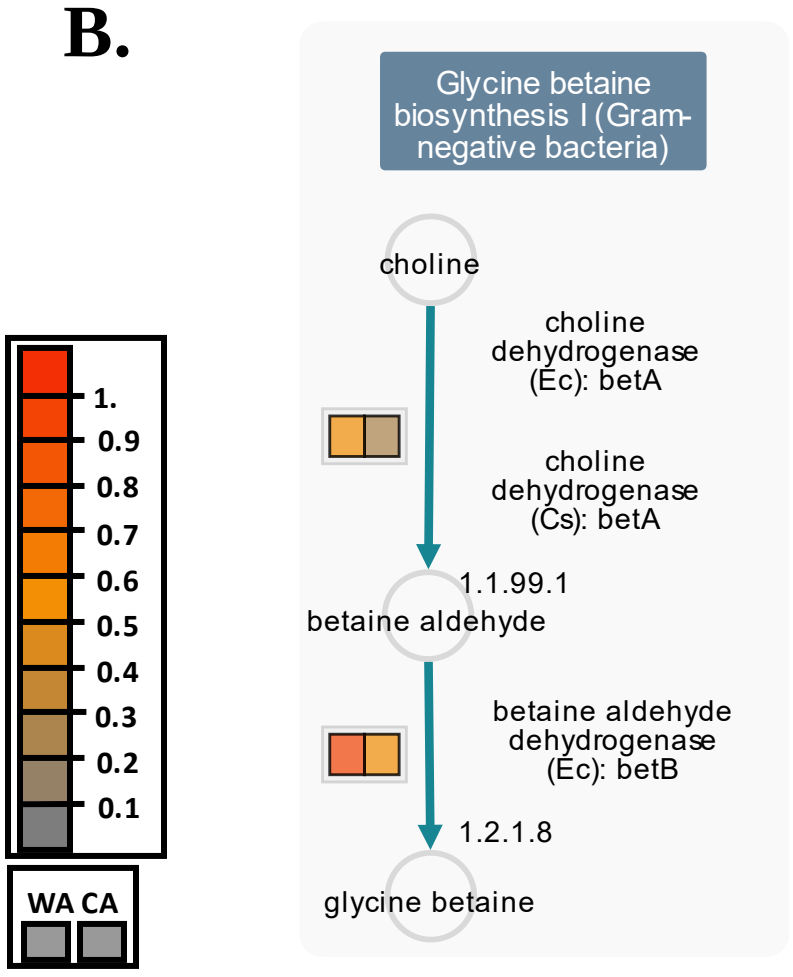

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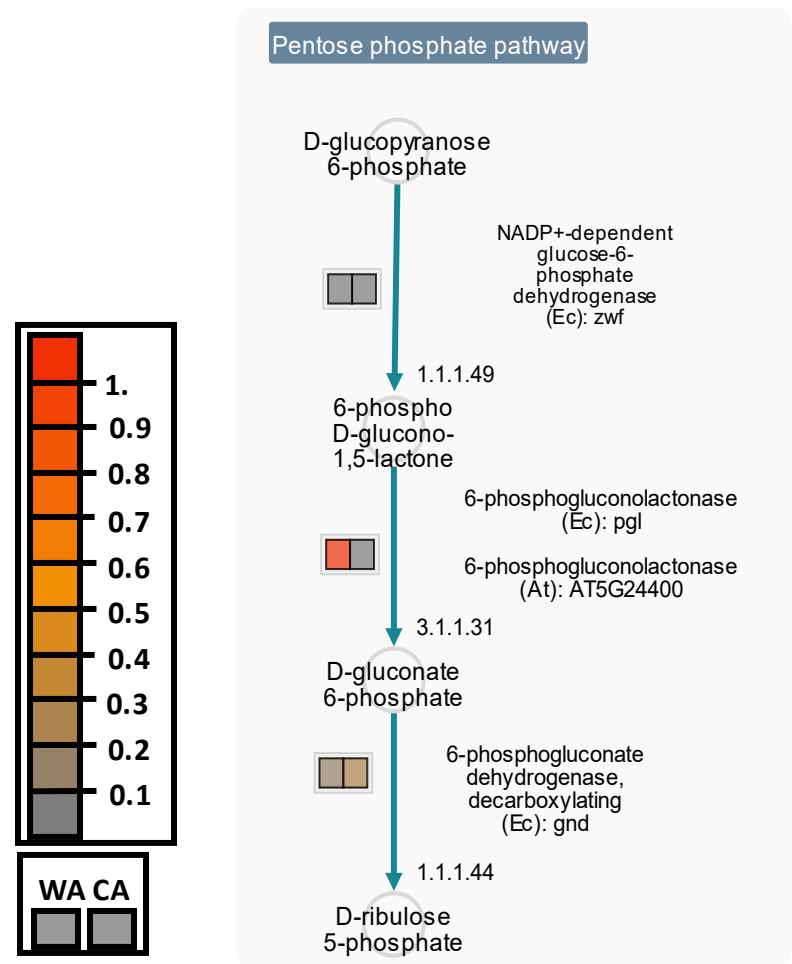

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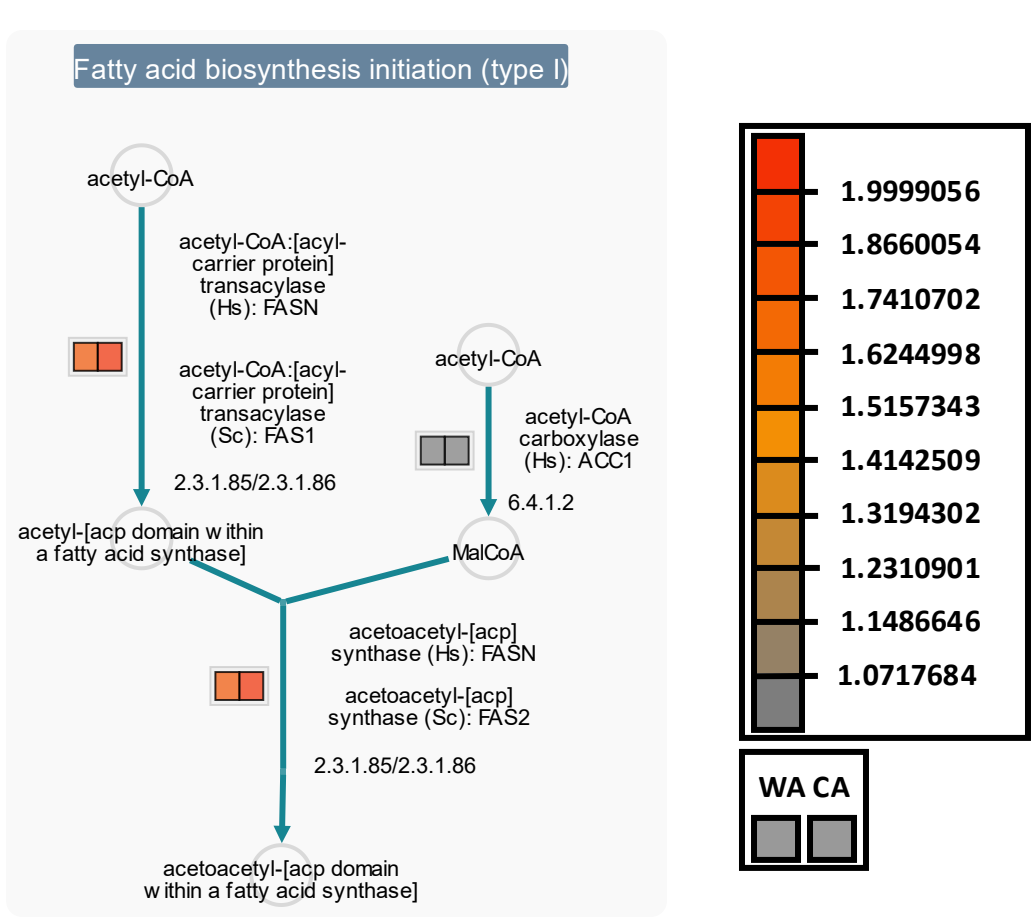

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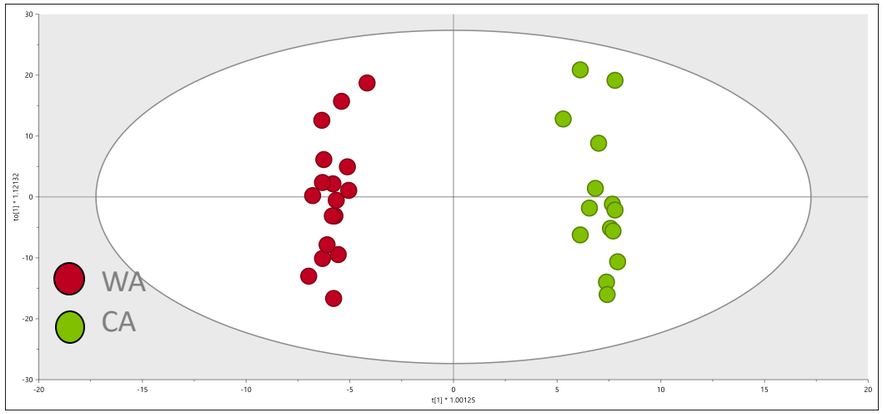

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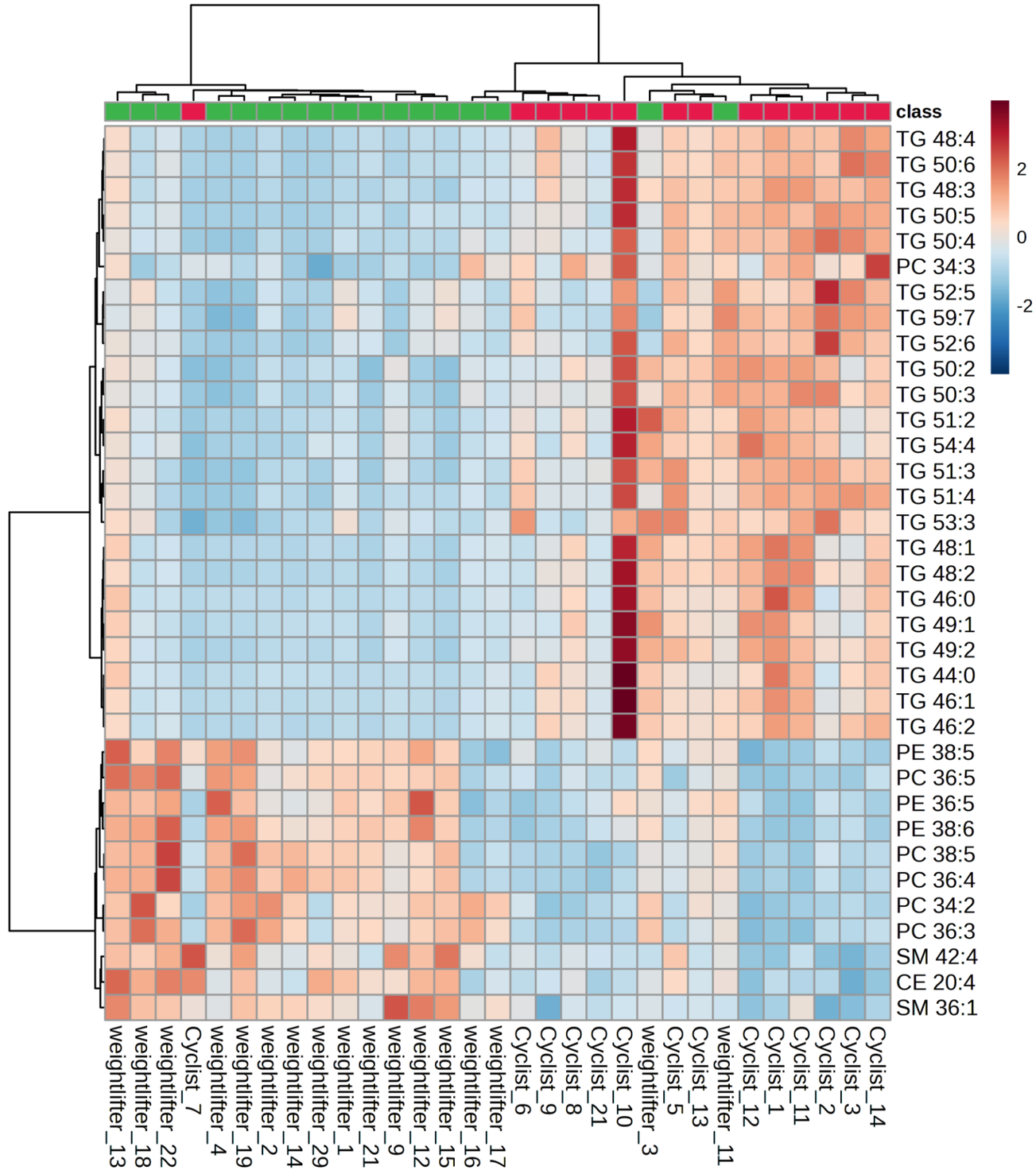

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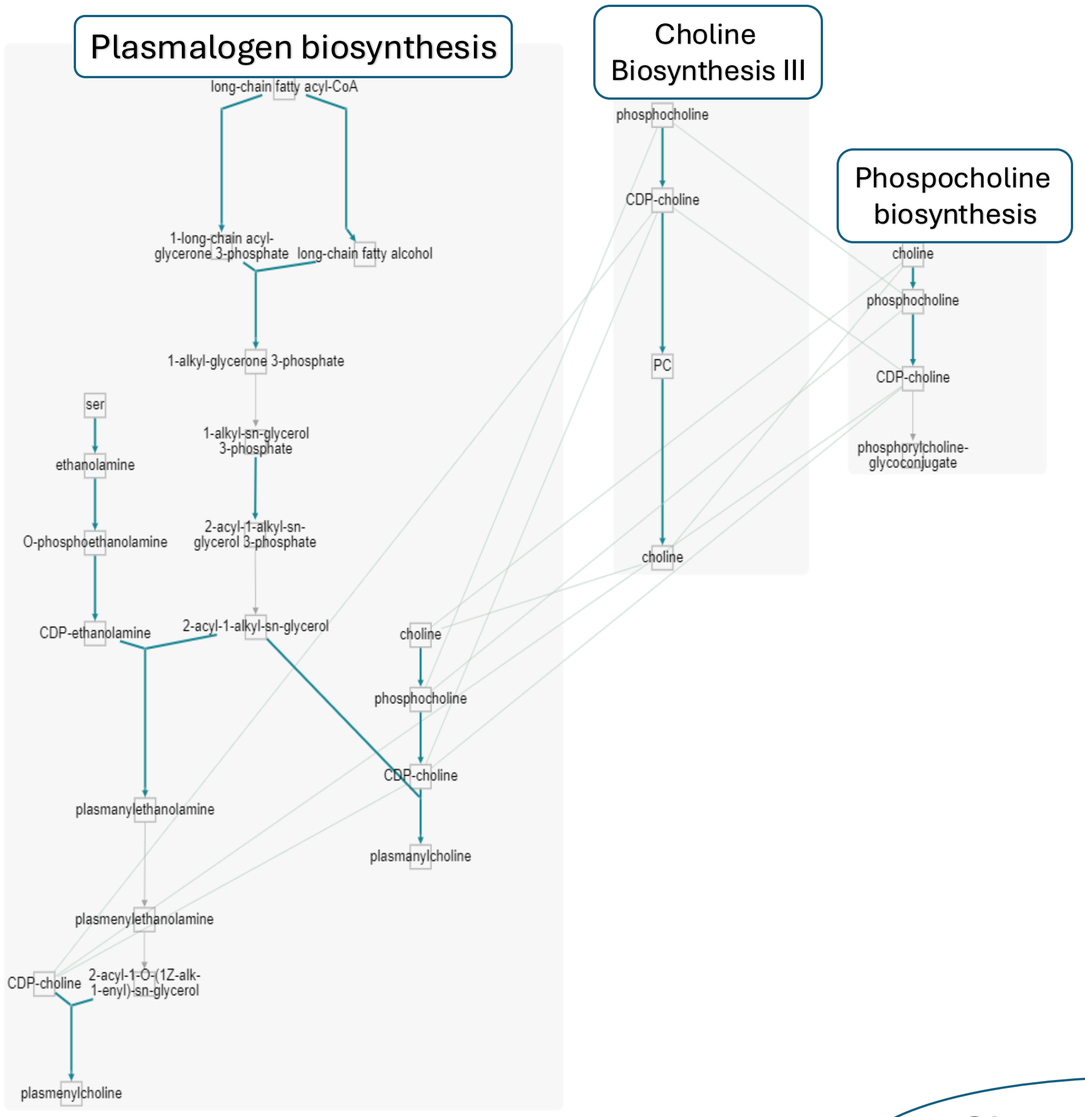

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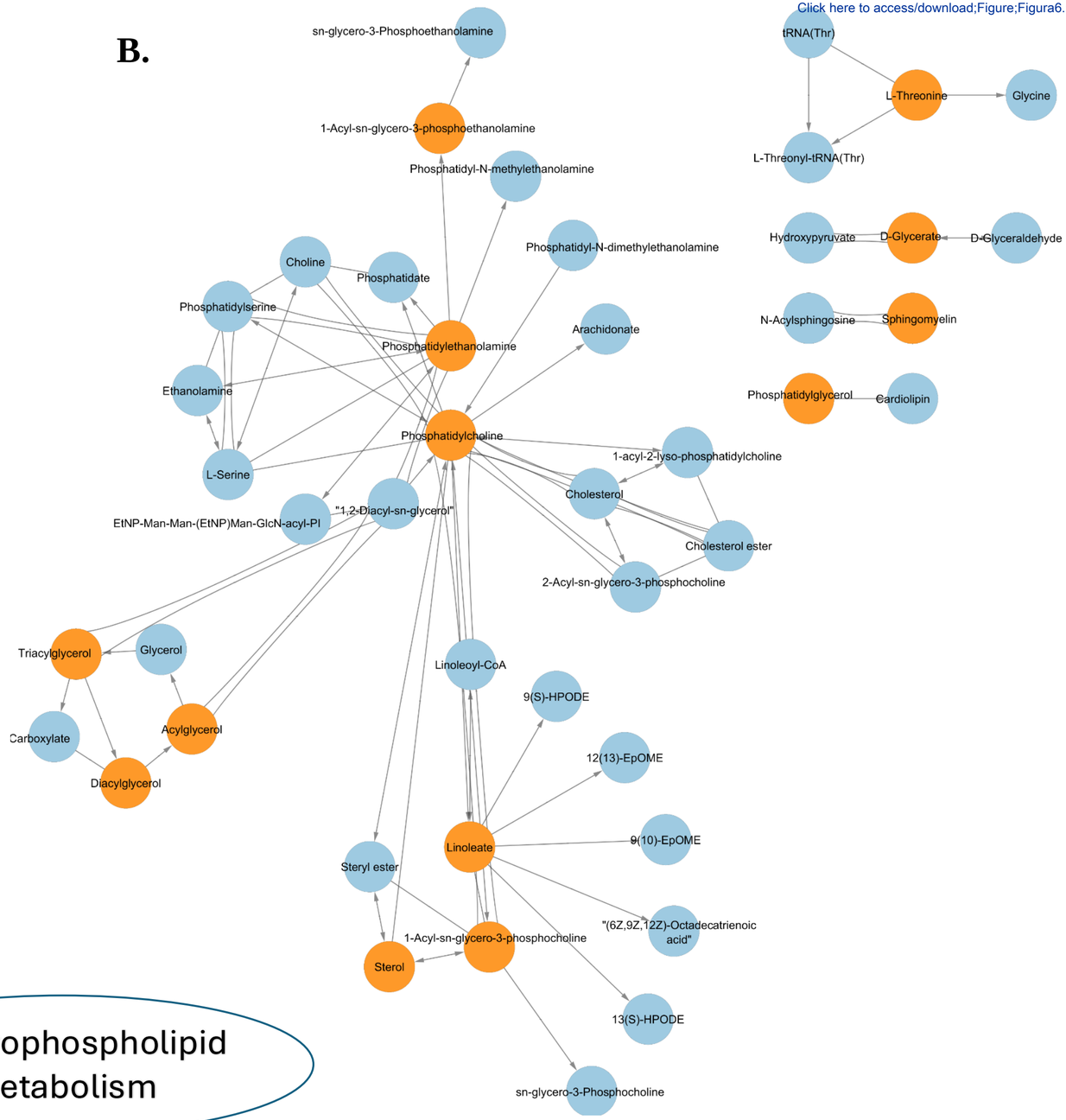

C.

Microbiome

Fatty Acid and Lipid Biosynthesis Pathways

Glycerophospholipid metabolism

Human Metabolome/Lipidome

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Supplementary file

Table S1 -S3 .xlsx

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Supplementary file

Table S4-S6.xlsx

9. CONCLUSIONES

Capítulo 1:

- Se evidencia la necesidad de investigaciones más amplias y detalladas que exploren la relación entre el ejercicio físico y la composición del microbioma intestinal en diferentes grupos de edad, incluidos adultos mayores de 65 años. Estas investigaciones deberían incluir no solo la diversidad y abundancia de microorganismos, sino también su función y las vías metabólicas involucradas, lo que podría arrojar luz sobre los efectos beneficiosos del ejercicio en la salud metabólica y el rendimiento físico.

- La falta de ensayos clínicos controlados en el campo del ejercicio y la microbioma intestinal representa una limitación significativa en la comprensión de esta relación. La mayoría de los estudios disponibles son observacionales, lo que dificulta establecer conclusiones sólidas sobre la causalidad entre el ejercicio y los cambios en la microbioma. Se insta a realizar ensayos controlados aleatorizados que permitan investigar de manera más precisa los efectos del ejercicio sobre el microbioma intestinal.

- La inclusión de enfoques multiómicos en futuras investigaciones, que integren datos de microbioma, genómica, transcriptómica, metabolómica, entre otros, podría proporcionar una visión más completa y detallada de los efectos del ejercicio sobre la salud y el rendimiento físico. Esta aproximación permitiría identificar no solo cambios en la composición microbiana, sino también en las funciones y rutas metabólicas asociadas.

- Es fundamental que las futuras investigaciones en este campo consideren aspectos del estilo de vida, como la dieta, el sueño y el ritmo circadiano, además del nivel de actividad física, para obtener una comprensión más completa de la relación entre el ejercicio y la microbioma intestinal. Integrar estos factores permitiría discernir mejor los mecanismos subyacentes y optimizar las intervenciones destinadas a mejorar la salud digestiva a través del ejercicio físico.

Capítulo 2:

- A lo largo de este capítulo, aportamos datos iniciales sobre el microbioma intestinal en atletas y no atletas colombianos.

- Utilizando técnicas como la metagenómica, se exploró la composición, diversidad y estructura del microbioma intestinal en poblaciones con diversas actividades físicas, destacando las posibles firmas microbianas asociadas con prácticas atléticas específicas. Se reportaron además resultados novedosos con respecto a miembros del microbioma deferentes a bacterias, tales como arqueas y virus.

- Se identificaron diferencias sustanciales en el microbioma intestinal entre ciclistas, levantadores de pesas y no atletas, resaltando 46 especies con características de abundancia distintivas, particularmente enfatizando las variaciones entre ciclistas y levantadores de pesas.

- Reconociendo limitaciones como la falta de datos sobre hábitos alimenticios y composición corporal individual, las investigaciones futuras deben incorporar estas variables para fortalecer las conclusiones. Se necesita más investigación para desentrañar las complejas relaciones entre los aspectos fisiológicos de diferentes actividades y el microbioma intestinal, contribuyendo a una comprensión más profunda del estilo de vida, la fisiología y las comunidades microbianas en el cuerpo humano.

- Futuros estudios, se verían beneficiados al incluir y correlacionar datos de la condición física de los sujetos (tales como consumo máximo de oxígeno, fuerza muscular) con datos de abundancia y composición de la microbioma intestinal.

Capítulo 3:

- Este capítulo reveló diferencias significativas en los perfiles metabólicos entre levantadores de pesas y ciclistas, lo que sugiere que el tipo de actividad física influye en el metabolismo tanto a nivel sistémico como microbiano. Los levantadores de pesas mostraron un enriquecimiento en metabolitos relacionados con el metabolismo de aminoácidos esenciales y nucleótidos, mientras que los ciclistas exhibieron perfiles más diversificados de ácidos grasos y una mayor producción cuerpos cetónicos lo que podría estar relacionado con la eficiencia en la producción de energía y la resistencia física.

- Análisis de integración entre datos metagenómicos y metabolómicos permitieron observar una correlación potencial entre las rutas metabólicas microbianas y los metabolitos del huésped, lo que refuerza la hipótesis de que la microbiota intestinal puede jugar un papel clave en la modulación del rendimiento deportivo y la salud metabólica. Sin embargo, estas asociaciones son, en gran parte, hipotéticas y necesitan ser validadas en estudios futuros con un enfoque multiómico más profundo.

- Si bien este estudio proporciona una visión preliminar sobre la relación entre el microbioma intestinal y el metabolismo en deportistas, la falta de datos sobre la dieta, el consumo de suplementos, mediciones antropométricas y variables fisiológicas limita la interpretación completa de los hallazgos. Es necesario realizar investigaciones adicionales que incluyan estas variables para contextualizar mejor las interacciones microbioma-metabolismo en respuesta al ejercicio físico.

- Los resultados presentados en este capítulo subrayan la importancia de emplear aproximaciones integrativas que combinen datos de metagenómica y metabolómica para obtener una visión más completa de cómo el ejercicio físico modula tanto la composición del microbioma como los perfiles metabólicos del huésped. Este enfoque permitirá identificar rutas metabólicas clave y metabolitos asociados con el rendimiento deportivo y la adaptación al ejercicio, proporcionando una base sólida para intervenciones futuras dirigidas a optimizar la salud y el rendimiento en atletas.

- Es esencial continuar investigando el impacto del ejercicio sobre el microbioma intestinal utilizando ensayos controlados que permitan evaluar de manera más precisa las modificaciones en la composición y función del microbioma. Además, sería valioso explorar la interacción entre otros factores del estilo de vida, como la dieta y el sueño, con el microbioma y la respuesta metabólica al ejercicio. De igual manera, la inclusión de otros dominios microbianos como virus, hongos y arqueas podría enriquecer la comprensión de estas interacciones y su impacto en la salud deportiva.

- La incorporación de otros enfoques ómicos, podría contribuir a la investigación en esta área del conocimiento.

CONCLUSIONES GENERALES DE LA TESIS

Esta tesis ha proporcionado una visión integral de la interacción entre el microbioma intestinal y la actividad física, utilizando un enfoque multidimensional que abarcó desde la teoría hasta la aplicación de metodologías avanzadas como la metagenómica y la metabolómica. A través de los tres capítulos, se logró identificar cómo la actividad física, tanto en atletas de alto rendimiento como en la población general, modula de manera significativa la composición y función del microbioma intestinal.

En el primer capítulo, se evidenció la influencia del nivel de actividad física en la diversidad y estructura microbiana, destacándose las diferencias clave entre atletas y no atletas. Estos hallazgos fueron ampliados en el segundo capítulo, donde se encontraron firmas microbianas específicas entre ciclistas y levantadores de pesas, lo que sugiere que las diferentes demandas energéticas de cada disciplina deportiva pueden impactar de forma diferencial en el microbioma. Finalmente, en el tercer capítulo, se demostró que estas variaciones en la composición microbiana también se reflejan en el perfil metabólico, con implicaciones potenciales en el rendimiento físico, particularmente en el metabolismo de ácidos grasos y aminoácidos.

A lo largo de esta investigación, se lograron identificar varias metodologías que van desde revisiones teóricas hasta enfoques ómicos, aportando evidencia novedosa sobre la relación entre la actividad física y el microbioma intestinal en una población de deportistas colombianos. Este estudio aporta una contribución valiosa al ser uno de los primeros en explorar esta interacción en dicha población, proporcionando una base útil para futuras investigaciones.

En resumen, esta tesis refuerza la idea de que la actividad física no solo influye en la composición del microbioma intestinal, sino también en sus funciones metabólicas, lo que puede tener implicaciones directas en el rendimiento deportivo y la salud metabólica. Estos hallazgos abren la puerta a nuevas líneas de investigación que podrían explorar más a fondo las interacciones causales y los mecanismos biológicos subyacentes mediante el uso de ensayos clínicos controlados e integración multiómica.

El campo de la interacción entre el microbioma intestinal y la actividad física ofrece amplias oportunidades para futuras investigaciones. Se prevé un enfoque más detallado en el estudio de los mecanismos causales que vinculan la actividad física con las modificaciones en la composición y función del microbioma. Esto implicará la implementación de ensayos clínicos controlados, que permitan establecer relaciones más claras y directas entre el tipo, la intensidad y la duración de la actividad física y los cambios en el microbioma.

Además, la integración de enfoques multiómicos, que combinen metagenómica, metabolómica, transcriptómica y proteómica, permitirá explorar con mayor profundidad las complejas interacciones entre la microbiota, el metabolismo del hospedador y las respuestas fisiológicas al ejercicio. Este enfoque ayudará a identificar biomarcadores específicos relacionados con el rendimiento físico y la salud metabólica,

con potenciales aplicaciones tanto en el ámbito deportivo como en la prevención y tratamiento de enfermedades metabólicas.

Finalmente, será fundamental incluir poblaciones diversas y realizar estudios longitudinales que aborden no solo la actividad física, sino también factores como la dieta, el sueño y el estrés, para obtener una visión más completa de cómo estos elementos interactúan con la microbiota intestinal y contribuyen al bienestar general.

10. PERSPECTIVAS

- Existe una necesidad imperante de ampliar el alcance de las investigaciones sobre el microbioma intestinal y el metabolismo en poblaciones atléticas y no atléticas. Futuros estudios podrían incluir una gama más amplia de deportes, como deportes de resistencia, deportes de equipo y actividades recreativas, así como también investigar diferentes grupos etarios y condiciones de salud. El uso de muestras más grandes y diversas permitirá capturar la complejidad y variabilidad de las respuestas metabólicas y del microbioma intestinal, así como las interacciones entre estos factores.

- Para lograr una comprensión más completa de la relación entre el ejercicio, el metabolismo y el microbioma intestinal, es esencial considerar variables adicionales, como la dieta, el consumo de suplementos, las mediciones antropométricas y variables fisiológicas. La integración de estos datos no solo enriquecerá el análisis, sino que también ayudará a identificar posibles factores de confusión que podrían influir en los resultados. Además, el uso de cuestionarios estandarizados y herramientas de evaluación de hábitos alimenticios podría proporcionar información valiosa sobre las prácticas dietéticas de los participantes.

- Es crucial investigar cómo los diferentes suplementos dietéticos y ergogénicos impactan la composición y función del microbioma intestinal, así como su relación con el rendimiento físico. La comprensión de estas interacciones podría ofrecer estrategias nutricionales personalizadas para optimizar el rendimiento deportivo y la salud metabólica.

- Futuros estudios deberían explorar la diversidad microbiana más allá de las especies bacterianas, incluyendo la caracterización de virus, hongos y arqueas en relación con el ejercicio y el metabolismo. Esto podría revelar interacciones complejas entre diferentes dominios microbianos y su impacto en la salud y el rendimiento, enriqueciendo así el conocimiento sobre el ecosistema microbiano humano.

- Se requiere una mayor investigación en vías metabólicas específicas para comprender mejor los mecanismos subyacentes de las adaptaciones metabólicas al ejercicio. Identificar las vías metabólicas clave involucradas en la respuesta al ejercicio permitirá el desarrollo de estrategias más precisas para mejorar el rendimiento deportivo y la salud metabólica.

- Es fundamental validar y reproducir los hallazgos actuales en diferentes cohortes y contextos para garantizar la fiabilidad y la generalización de los resultados. La replicación de estudios en diversas poblaciones y condiciones ayudará a confirmar la robustez de las asociaciones observadas y a identificar posibles variaciones interindividuales.

- La implementación de estudios longitudinales que analicen los cambios en el microbioma intestinal a lo largo del tiempo y en respuesta a intervenciones de ejercicio específicas será fundamental. Esto permitirá evaluar no solo los efectos inmediatos del ejercicio sobre el microbioma, sino también los cambios adaptativos que ocurren a largo plazo y su relación con la salud metabólica.

- La integración de técnicas de análisis avanzadas, como la secuenciación de alto rendimiento y el análisis de vías metabólicas, permitirá una comprensión más profunda de la interacción entre el microbioma intestinal, el metabolismo y el ejercicio. Estas herramientas analíticas proporcionarán información detallada sobre los mecanismos moleculares subyacentes y las vías metabólicas implicadas.

- La investigación en este campo no solo tiene implicaciones para el rendimiento deportivo, sino también para la salud pública. Comprender cómo el ejercicio y el microbioma influyen en el metabolismo podría ayudar a diseñar intervenciones dirigidas a mejorar la salud metabólica en poblaciones diversas, incluyendo aquellas con riesgo de enfermedades metabólicas.

11. PRODUCTOS DE LA TESIS

Los artículos se encuentran a lo largo del documento, los anexos correspondientes a la información suplementaria se encuentran en la carpeta de anexos de artículos divididos en carpetas por artículo. Todos los productos que se mencionarán a continuación se encuentran en la carpeta de anexos de productos de la tesis divididos según los numerales a continuación:

11.1: ARTÍCULOS CIENTIFICOS

- **Artículo 1: Aya V,** Flórez A, Perez L, Ramírez JD. Association between physical activity and changes in intestinal microbioma composition: A systematic review. PLOS ONE. 2021 Feb 25;16(2):e0247039.

- **Artículo 2: Aya V,** Jimenez P, Muñoz E, Ramírez JD. Effects of exercise and physical activity on gut microbioma composition and function in older adults: a systematic review. BMC Geriatr. 2023 Jun 12;23(1):364.

- **Artículo 3: Aya V,** Vega LC, Muñoz E, Muñoz M, López DF, Guzmán MP, et al. Divergent Gut Microbioma: Archaeal and Bacterial Signatures Unveil Unique Patterns in Colombian Cyclists Compared to Weightlifters and Non-Athletes. Adv Biol. n/a(n/a):2400069.

- **Artículo 4: Aya V**, Pardo D, Vega LC, Cala MP, Ramírez JD. Integrating Metagenomics and Metabolomics to Study the Gut Microbiome and Host Relationship in Sports Across Different Energy Systems. (Sometido a Sports Medicine)

11.2 PRESENTACIÓN EN EVENTOS

Octubre/2023 **Tunja, Colombia**	**"I Congreso Internación De Medicina Del Deporte y El Ejercicio En Altura" Y** **"I Congreso Internacional De Tendencias Del Entrenamiento Deportivo Y La Actividad Física**" **Tipo de evento:** Congreso **Ámbito:** Nacional **Nombre del producto:** "Interacción entre el microbioma intestinal y la actividad física: implementación de ciencias ómicas en áreas del deporte y la actividad física" **Tipo de producto:** Presentación oral
Febrero/2024 **Sevilla, España**	**XV Workshop de la Sociedad Española de Microbioma, Probióticos y Prebióticos** **Tipo de evento:** Workshop **Ámbito:** Internacional

	Nombre del producto: "Caracterización de la microbioma intestinal de deportistas profesionales colombianos: halterofilia y ciclismo de ruta." **Tipo de producto:** Comunicación oral
	XV Workshop de la Sociedad Española de Microbioma, Probióticos y Prebióticos **Tipo de evento:** Workshop **Ámbito:** Internacional **Nombre del producto:** "Diversidad y abundancia relativa de especies de Prevotella intestinal en ciclistas y no deportistas colombianos" **Tipo de producto:** Póster
Septiembre/2024 **Bogotá, Colombia**	**IV Congreso Internacional de Entrenamiento Deportivo Pedagogía y Administración Deportiva** **Tipo de evento: Congreso** **Ámbito:** Nacional **Nombre del producto:** "Nuevas Perspectivas en la Fisiología del Deporte: Integración de ciencias ómicas para el estudio de población deportiva". **Tipo de producto:** Ponencia

11.3 PASANTÍA

Bogotá, Colombia/ Mayo – Noviembre 2023	"Entrenamiento en la obtención y análisis de datos metabolomomicos y lipidomicos" **Pasantía doctoral** MetCore - Metabolomics Core Facility, Vice-Presidency for Research, Universidad de los Andes, Bogotá, Colombia **Duración:** 6 meses

11.4 BECAS Y RECONOCIMIENTOS

Julio/2019 Bogotá/Colombia	**Beca asistente graduado/estudiante de doctorado** Facultad de Ciencias Naturales
	Small Grant

12. BIBLIOGRAFÍA

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