UNIVERSITAT DE BARCELONA

Efecto de la suplementación aguda con zumo de remolacha en el rendimiento aeróbico y anaeróbico

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Escola de Doctorat

PROGRAMA DE DOCTORADO: BIOMEDICINA

UNIVERSIDAD DE BARCELONA FACULTAD DE BIOLOGIA

DEPARTAMENTO DE BIOLOGÍA CELULAR, FISIOLOGÍA E INMUNOLOGÍA

EFECTO DE LA SUPLEMENTACIÓN AGUDA CON ZUMO DE REMOLACHA EN EL RENDIMIENTO AERÓBICO Y ANAERÓBICO

Memoria presentada por

Eduardo Cuenca García

Para optar al grado de:

Doctor por la Universitat de Barcelona

Director Dr. Manuel Vicente Garnacho Castaño.

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Esta tesis, como a lo largo de mi vida deportiva donde siempre he competido en pruebas combindas, ha sido una competición contra mi mismo. Durante estos cuatro años he ido preparando cada intervención, cada revisión y artículo como si fuera la competición más importante de mi vida, donde no solo un artículo era importante sino la sumatoria de todo el trabajo realizado, al igual que cuando preparaba una competición en decatlon, muchas personas han estado ayudándome desde el principio, a través de su conocimiento, correcciones, apoyo, felicitaciones y sobre todo ilusión en este proyecto.

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Finalizo con mi amor incondicional, Teba, nuestro camino no ha sido fácil durante este tiempo, eres mi ejemplo a seguir de superación, gracias por aguantarme y apoyarme.

"LAS COSAS PASAN POR ALGO"

SINOPSIS

This PhD thesis sought to gain insight into the effects of acute supplementation with beetroot juice (BJ) on high intensity anaerobic performance and on cardiorespiratory performance when executing prolonged, mostly aerobic, efforts in different sports.

The high inorganic nitrate content of BJ makes this supplement a nutritional ergogenic aid aimed at enhancing performance and backed by high scientific evidence. Once metabolized in the organism, nitrate is converted into nitric oxide with its numerous physiological functions.

After several reviews and experimental studies we found the following results. When reviewing the impacts of BJ on anaerobic performance, we only identified 9 studies that met the exclusion criteria highlighting the lack of studies examining the effects of this supplement on high intensity efforts in fairly well-trained athletes. During our first experimental intervention in triathletes, no significant improvement was observed in the cardiorespiratory variables examined, although there was improvement in the second ventilatory threshold (VT2) time but the difference lacked significance. In a second and third study in which we assessed high-intensity anaerobic efforts, improvements were noted in peak power, average power and lactate levels while no effects were detected on muscular fatigue.

In conclusion, BJ supplementation did not improve performance in prolonged aerobic efforts in highly trained triathletes. However, the slight non-significant improvement noted in VT2 time raises several questions, as the mechanical and physiological mechanisms addressed so far are still poorly understood. The acute intake of BJ supplements had an ergogenic effect on performance in a high-intensity anaerobic effort. These improvements could indicate greater performance in high-intensity modalities and those requiring maximal power or acceleration levels due to possible specific beneficial effects on power output and blood flow produced in type II motor units. In contrast, BJ was not able to diminish muscular fatigue during a high-intensity effort.

There is controversy in the scientific literature over the beneficial effects of BJ in high-intensity efforts. Recommendations for BJ supplementation are its acute intake 2-3 hours before the exercise activity at a dose between 6.4 and 12 mmol depending on the athletes' physical condition.

ÍNDICE

LISTADO DE ABREVIATURAS

ATP: adenosintrifosfato

CMJ: salto en contramovimiento

CO_2: Dióxido de carbono.

eNOS: enzima endotelial óxido nítrico sintasa

FEO_2: concentración de oxígeno en el aire espirado.

H: altura de vuelo.

JCR: journal citation reports

Kp: kilopondios

NO: óxido nítrico

NO_2^-: Nitrito

NO_3^-: Nitrato inorgánico.

O_2: Oxígeno.

PCr: fosfocreatina.

PaCO2: presión parcial de dióxido de carbono.

PETO2: presión parcial del oxígeno al final de la espiración.

PC: potencia pico.

PM: potencia media.

RER: tasa de intercambio respiratorio.

Ua: Umbral aeróbico.

Uan: Umbral anaeróbico.

UE: Umbral de electromiografía

UL: Umbral de lactato

VCO_2: Volumen dióxido de carbono

VE: Ventilación pulmonar

VT: Umbral de ventilación.

VT1: primer umbral ventilatorio

VT2: segundo umbral ventilatorio

VO_2: Consumo de oxígeno

VO_{2max} : Consumo máximo de oxígeno

W: vatios.

W_{med}: potencia media

W_{pico}: potencia pico

ZM: zumo de remolacha

1. INTRODUCCIÓN

1.1 Contextualización.

La presente tesis doctoral está fundamentada en el trabajo realizado desde el año 2016, con la participación en dos congresos (Anexo 1, 2) y la publicación de 4 artículos en Journal Citaton Report (JCR) de primer cuartil. Además, se ha realizado una búsqueda previa en la literatura científica sobre los efectos del zumo de remolacha (ZM) en el rendimiento de deportes de resistencia.

La importancia que está adquiriendo la suplementación con nitratos procedentes del ZR y, por ende, de su transformación final en óxido nítrico (NO), en el rendimiento de modalidades deportivas que implican el metabolismo aeróbico y anaeróbico, hace imprescindible investigar la influencia del ZR en el deporte. La igualdad en el deporte de alta competición exige que hasta el más mínimo detalle, como la mejora de un 0,6% en el rendimiento (1), deba de tenerse en consideración. Además de una correcta planificación deportiva, las ayudas ergonutricionales son uno de los eslabones determinantes para que todos los mecanismos fisiológicos funcionen correctamente y se optimice el rendimiento (2).

El Instituto Australiano del Deporte (3) clasifica las ayudas ergonutricionales para el rendimiento deportivo en base a una evidencia científica demostrada. El ZR es considerando por este organismo como una ayuda ergonutricional de alta evidencia científica, que incrementa el rendimiento deportivo. Sin embargo, la controversia generada por las diversas investigaciones sobre su potencial efecto ergogénico, hace necesario seguir indagando para determinar los mecanismos fisiológicos subyacentes de la suplementación con ZR.

1.2 Esfuerzos de resistencia y esfuerzos de alta intensidad

Hay que considerar que el efecto de un determinado suplemento sobre el rendimiento puede ser específico de cada modalidad deportiva (4), en función de las necesidades metabólicas y/o mecánicas de las mismas, habiendo suplementos que pueden tener un efecto ergogénico ante determinados tipos de esfuerzos y no ante otros de naturaleza metabólica diferente.

Por ello, los dos tipos de esfuerzo seleccionados para estudiar durante esta tesis son: esfuerzos prolongados denominados de *"resistencia"* que implican mayoritariamente un metabolismo aeróbico, *y* de corta duración, distinguidos como esfuerzos de *"alta intensidad",* en los que está implicado prioritariamente el metabolismo anaeróbico.

1.3 Esfuerzos de resistencia predominantemente aeróbicos

Estos esfuerzos se caracterizan por ser prolongados en el tiempo e implicar un metabolismo prioritariamente aeróbico, donde la aportación y el comportamiento del consumo de oxígeno (VO_2) y de los parámetros ventilatorios a determinadas intensidades será determinante para mejorar las expectativas de éxito en las pruebas (triatlón, pruebas de resistencia atlética, natación, ciclismo, etc). Por tanto, el rendimiento vendrá determinado por aquellos parámetros que condicionen este metabolismo e incluso de cómo el atleta tenga desarrollado el metabolismo de transición aeróbico-anaeróbico. Se ha establecido que el rendimiento en el ejercicio de resistencia está relacionado con los siguientes parámetros: una mayor absorción de oxígeno o volumen máximo de oxígeno (VO_{2max}), el umbral de lactato (UL), el umbral de ventilación (VT), la economía /

eficiencia del ejercicio (5-10) y la cinética del consumo de oxígeno (11). Estas variables nos darán información sobre la capacidad funcional del deportista, de su estado de entrenamiento, así como de los posibles cambios en su planificación deportiva.

1.3.1 Los umbrales ventilatorios y las respuestas cardioventilatorias

El rendimiento deportivo en deportes de resistencia esta directamente relacionado con el VT, término fundamental de esta tesis durante esfuerzos aeróbicos. El concepto de VT surgió en 1927 al comprobar Heymans (12) que había una relación directa entre las concentraciones de lactato con la intensidad de la ventilación pulmonar durante el ejercicio físico. Este término muchas veces se identifica con el de UL, aunque en muchas ocasiones se refieran a un momento metabólico similar, independientemente de la metodología de evaluación utilizada. Ambos términos refuerzan las bases fisiológicas para entender lo que se conoce como transición aeróbica–anaeróbica, claves importantes en el rendimiento deportivo de los esfuerzos aeróbicos prolongados.

Skinner y Maclellan, (extraído de López y Fernández (13)) establecen las siguientes respuestas cardioventilatorias en tres fases diferenciadas durante el ejercicio incremental de resistencia:

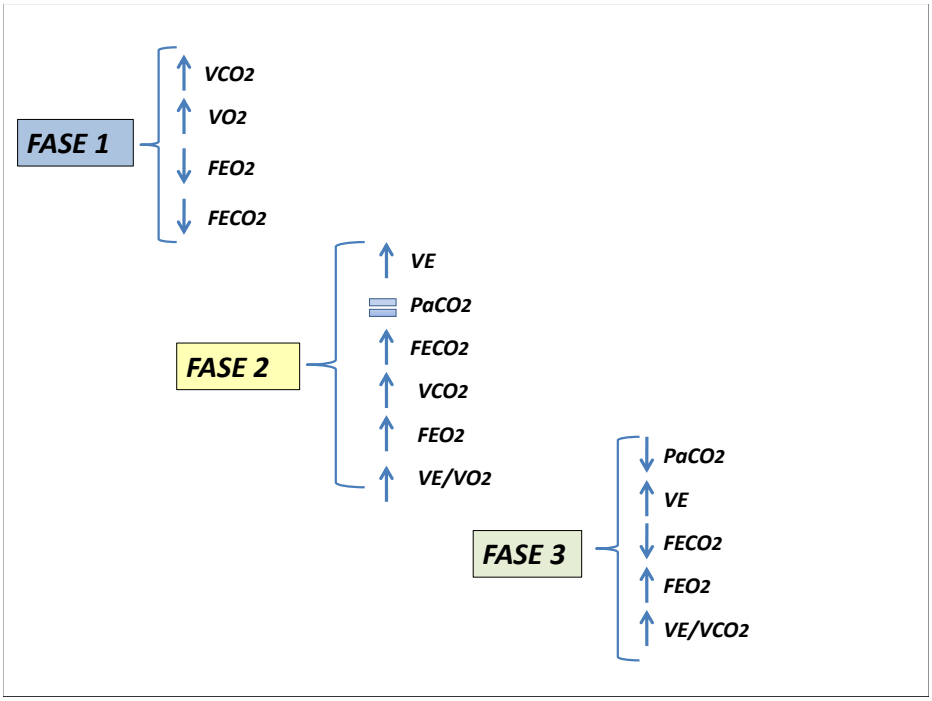

Figura 1. *Cambios en las variables cardioventilatorias durante una prueba incremental. VCO_2: volumen de dióxido de carbono; VO_2: Consumo de oxígeno; FEO_2: concentración de oxígeno en el aire expirado; $FECO_2$: fracción expirada de dióxido de carbono; VE: ventilación pulmonar; $PaCO_2$: presión parcial dióxido de carbono; VE/VO_2: equivalente ventilatorio de oxígeno.*

Fase 1: Amortiguación del lactato con producción de dióxido de carbono (VCO_2) en relación con el VO_2. Aquí observamos que a bajas intensidades la cantidad de oxígeno extraída de los tejidos aumenta, existiendo una menor concentración de oxígeno en el aire espirado (FEO_2), un aumento de la tasa de producción de VCO_2 y una disminución de la fracción expirada de CO_2 ($FECO_2$). Durante esta fase la producción de adenosintrifosfato (ATP), proviene fundamentalmente del metabolismo aeróbico u oxidativo.

Fase II: Incremento de la ventilación pulmonar (VE), proporcional al aumento del VCO_2, mientras que la presión parcial de dióxido de carbono ($PaCO_2$) se mantiene relativamente constante. A medida que la intensidad del ejercicio aumenta, alrededor del 60% VO_{2max}, se reclutan un mayor número de fibras musculares tipo II. La producción de una mayor

cantidad de lactato es amortiguada por el sistema de bicarbonato (Ion bicarbonato HCO_3^-), aumentando de esta manera la $FECO_2$, existiendo un aumento del VCO_2, y un aumento de la FEO_2. La VE incrementa de forma no lineal respecto al VO_2 y al VCO_2. Se produce, por tanto, un aumento del equivalente ventilatorio del oxígeno (VE/VO_2).

Fase III: Se produce una compensación respiratoria de la acidosis metabólica, con descenso de la $PaCO_2$. A intensidades más altas la concentración de lactato es superior a los procesos de aclaramiento, aumentando la VE. De forma conjunta hay un descenso de la $FECO_2$, y un aumento de la FEO_2, elevando los valores del equivalente ventilatorio del VCO_2 (VE/VCO_2) que hasta entonces se mantenían estables.

El llamado *primer umbral ventilatorio* (VT1), esta causado por un aumento de la concentración arterial de lactato por encima de los niveles de reposo, que sirve para eliminar los niveles de exceso de CO_2 derivado de la amortiguación por el bicarbonato de la acidosis metabólica y aumenta la homeostasis de la $PaCO_2$. El aumento de intensidad por encima del VT1, provoca un aumento desproporcionado de la VE en relación al VCO_2, que se asocia a un aumento del VE/VCO_2 y un descenso de la $PaCO_2$. Este punto de inflexión metabólica se conoce como *segundo umbral ventilatorio* (VT2).

Siguiendo la figura 2 podremos definir el VT1 como la primera intensidad donde el ejercicio corresponde con una inicial pérdida de la linealidad de la VE, junto con el inicio del aumento continuado del VE/VO_2 y descenso FEO_2 o $P_{ET}CO_2$.

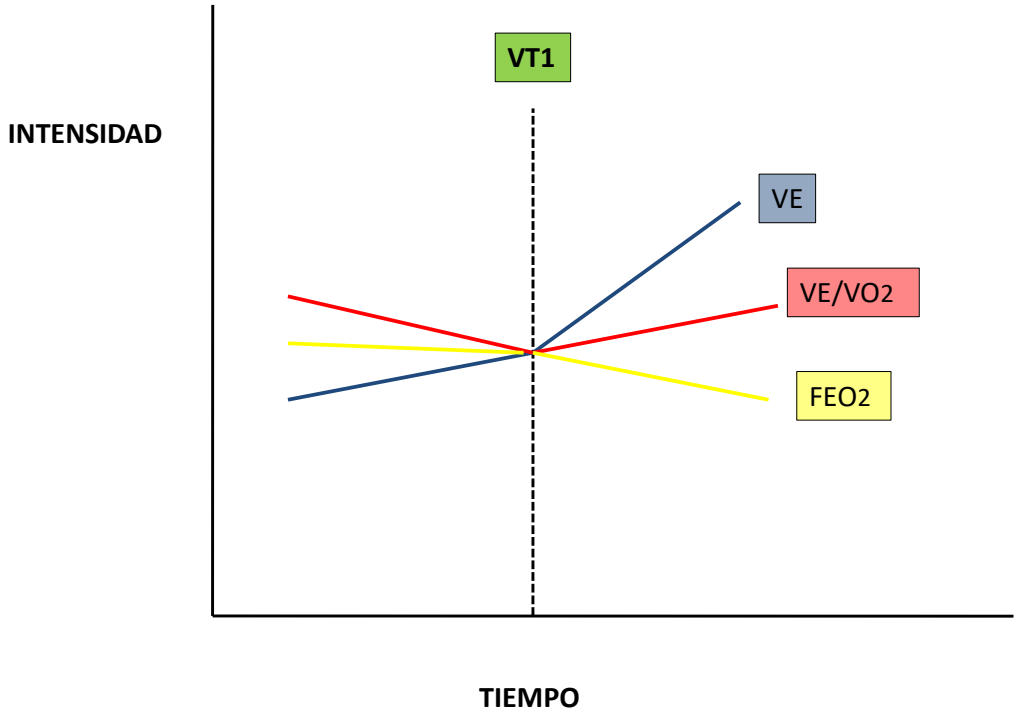

Figura 2. *Cambios en las variables ventilatorias en VT1.*

Mientras que el VT2 (figura 3), esta determinado por un segundo cambio no lineal de la VE y del VE/VO_2, junto con un marcado aumento continuado del VE/VCO_2, y un descenso continuo de la $FECO_2$, o de la $P_{ET}CO_2$.

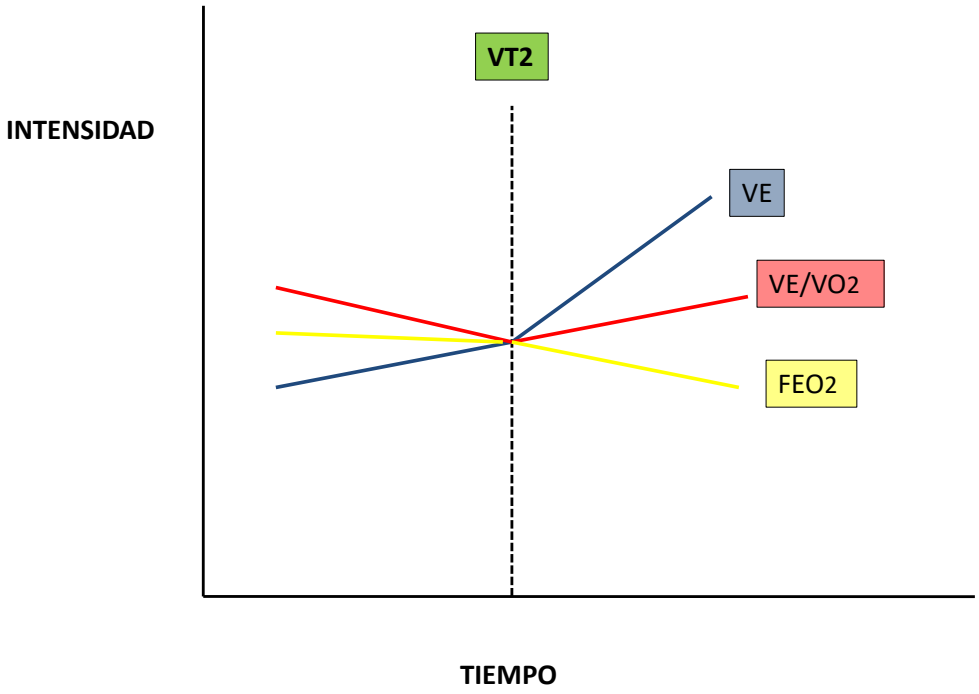

Figura 3. *Cambios en las variables ventilatorias en VT2*

Además de estas variables ventilatorias, debemos tener en cuenta la respuesta de la frecuencia cardiaca ante el ejercicio durante un test incremental, Conconi y cols.(14), situaba la transición aeróbico- anaeróbico como aquella zona donde existe un aumento curvilíneo de la frecuencia cardiaca respecto al incremento de velocidad (deflexión de la frecuencia cardiaca). Esta variable puede ser una herramienta para entrenadores que no tengan acceso a otro tipo de instrumentos y medidas.

Explicado lo que ocurre con las variables cardioventilatorias durante esfuerzos incrementales, faltaría comentar que ocurre con las diferentes intensidades (umbrales), durante una carga constante, ya que en deportes de resistencia donde la intensidad se

mantiene constante, el control de esta carga es un factor clave para el rendimiento. Al comienzo del ejercicio se produce un incremento rápido de la ventilación (VE), producción del O_2 y eliminicación del CO_2 para satisfacer las necesidades del organismo, tras 3-4 minutos, este incremento se realiza de forma más gradual hasta alcanzar VT1 (explicado anteriormente), si aumentamos esta intensidad provocaríamos un aumento desproporcionado de la VE en relación al VCO_2, un aumento del VE/VCO_2 y descenso de la $PaCO_2$, aproximándonos al VT2. Respecto al tiempo que podemos aguantar a carga constante ya sea en VT1 o VT2, además del control de las variables cardioventilarias citadas, existen otros mecanismos que podrían disminuir el tiempo en los diferentes umbrales como las alteraciones en el pH, alteraciones en la temperatura, acumulación de productos metabólicos, pérdida de la homeostasis, estrés oxídativo ó lesión muscular (15).

1.3.2 El componente lento del consumo de oxígeno

El componente lento del VO_2 se define como el incremento del VO_2 que ocurre en ejercicios prolongados a una intensidad de carga constante. En la figura 4, vemos el comportamiento de la cinética del VO_2, durante una prueba constante, el componente lento de oxígeno se localiza más allá del tercer minuto del ejercicio hasta la finalización de la misma (16), tras un primer cambio en el VO_2, observamos que se estabiliza aumentando de forma progresiva hasta terminar la prueba. Siguiendo a Billat y cols (17) el rendimiento en resistencia esta relacionado con la velocidad que puede alcanzar el deportista y el tiempo hasta el agotamiento estando directamente relacionado con las respuestas del VO_2.

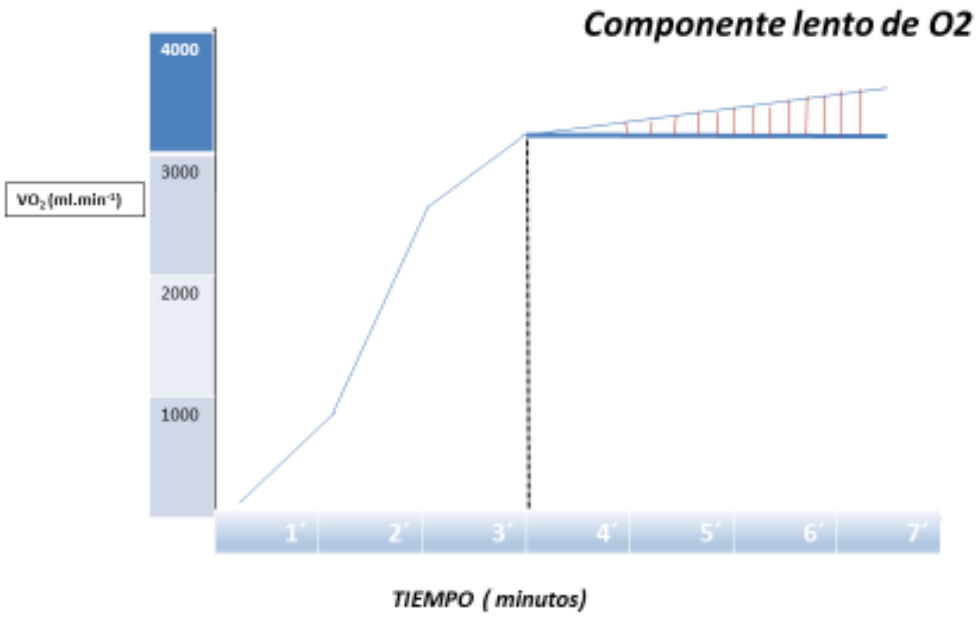

Figura 4. *Cinética del VO_2 durante una prueba constante y momento donde se identifica del componente lento de O_2.*

Hay diferentes parámetros que están estrechamente relaciones con este cambio, como el trabajo de la musculatura cardiorrespiratoria (16), el aumento de la temperatura (18), la aparición de ácido láctico (19), el reclutamiento de fibras tipo II (16). La aparición del componente lento del VO_2 se asocia a la producción de energía a través de las gulocolisis anaeróbica (20). El entrenamiento disminuye esta aparición del componente lento del VO_2, siendo un índice de tolerancia a la intensidad del ejercicio (21).

Sería muy útil incluir pruebas a carga constante para el control del componente lento del VO_2 además de la estandarizada prueba de esfuerzo incremental en la valoración fisiológica del deportista y la planificación de su entrenamiento deportivo.

1.3.3 La eficiencia mecánica y economía del esfuerzo.

Además del componente lento del VO_2, también se hablará sobre la eficiencia mecánica o también conocida como *gross eficiency* y la *economía del esfuerzo.* La eficiencia mecánica se define como la relación entre la potencia de pedaleo y el gasto de energía, factor determinante en ciclistas de resistencia (22-24) y en triatletas (25). Esta relación muestra que a mayor trabajo realizado con menor gasto energético el deportista es más eficiente (24). Esta eficiencia mecánica entre ciclistas entrenados puede variar entre un 18% y un 23,5%, estando directamente relacionado con un mayor porcentaje de fibras musculares tipo I (26). Este estudio coincide con los propuestos por Horowitz y cols. (24) y Coyle y cols. (26)[26]. Esta eficiencia puede mejorarse con el entrenamiento de fuerza (27,28).

Respecto a la economía del esfuerzo, se define como el VO_2 necesario que se utiliza a una determinada velocidad, es decir, los litros de oxígeno (O_2) que se utilizan durante el ejercicio. Al igual que pasaba con la eficiencia mecánica, el objetivo del entrenamiento deportivo es mejorar este parámetro, si conseguimos a una carga mayor de velocidad consumir los mismos litros de O_2, nuestros deportistas serán más económicos mejorando su rendimiento en la prueba. Hay diferentes parámetros a tener en cuenta que están directamente relacionados con la economía del esfuerzo, como por ejemplo la altura del

sillín (29), la cadencia (30), el entrenamiento de la fuerza (31) ó suplementación con quercetina (32).

1.4 Esfuerzos de alta intensidad predominantemente anaeróbicos

En las últimas décadas el termino anaeróbico ha constituido muchas idas y venidas en cuanto a su definición, siendo la más actual la denominada como independiente de oxígeno o no mitocondrial (33), llamándose vía de los fosfágenos (conocida como vía anaeróbica aláctica), y glucolisis (vía anaeróbica láctica) ya que aunque el oxígeno no esta directamente involucrado se encuentra presente.

Los esfuerzos de alta intensidad se definen como esfuerzos máximos con una duración de 6 segundos hasta 1 minuto, donde predomina principalmente la vía energética glucolítica, además de la vía de fosfágenos y fosforilación oxidativa (34). Durante este tipo de intensidades, cobran especial importancia el reclutamiento de unidades motoras tipo II.

1.4.1 El test de Wingate.

El test Wingate valora la potencia anaeróbica en deportistas y establece a través de un índice de fatiga, las respuestas a ejercicios supramaximales, así como la capacidad para mantener estos tipos de esfuerzos. Este se desarrolló en 1974, en el instituto de Wingate de Educación Física y Deportes en Israel. Se realiza en un cicloergómetro y la duración del test es de 30 segundos realizado a la máxima intensidad posible, con un calentamiento previo y una vuelta a la calma. Los datos que se obtienen son los siguientes:

- Tiempo que se tarda en llegar a la máxima potencia; este valor dependerá de la calidad de las fibras musculares; a una mayor cantidad de fibras musculares tipo II, se alcanzará antes los valores de potencia máxima que en sujetos con una mayor cantidad de fibras tipo I.
- Potencia máxima; este valor se puede expresar de forma pura en vatios (W), o como se ha desarrollado en esta investigación haciendo una ponderación en W/kg, para poder comparar entre sujetos. Siguiendo a Bar-OR (35), las cargas asignadas en kilopondios (Kp) correspondieron al 7,5 % de la masa corporal.
- El índice de fatiga, ya que la potencia va descendiendo a lo largo del test. Se define como el porcentaje de pérdida de potencia que tiene lugar durante el test.
- Potencia mínima de la prueba al finalizar la misma.

Existen diferentes estudios (25,36-39) donde se compara los resultados del test de Wingate con el rendimiento en multitud de especialidades deportivas. Estos estudios corroboran nuevamente la elección de este test como medidor directo para disciplinas deportivas donde los esfuerzos de alta intensidad sean predominantes. Sin embargo, hay que reseñar su falta de especificidad en la evaluación de muchas acciones motrices y modalidades deportivas anaeróbicas.

1.4.2 La fatiga metabólica y muscular

Siguiendo a García Manso y cols (40), se define la fatiga metabólica como una disminución de la capacidad de rendimiento, que aparece asociada a sobrecargas funcionales y que se manifiesta tras la ejecución de un ejercicio físico. La activación que se produce en la musculatura durante la práctica deportiva, hace que se vayan agotando las reservas energéticas para producir dicha activación, y dependiendo de la intensidad

del ejercicio se asociarán a un tipo de fibras musculares. Las fibras tipo I tienen una gran densidad capilar, conteniendo una mayor cantidad de mioglobina y mitocondrias, responsables de transportar oxígeno. Estas fibras musculares permiten un mayor ahorro energético y mayor resistencia a la fatiga. Mientras que las fibras tipo II, al carecer de mioglobina utilizan grandes cantidades de ATP, fatigándose muy rápido. Esta fatiga se asocia a la disminución de los niveles de calcio (41), el aumento de fosfatos inorgánicos e hidrógeno que hacen que disminuya la fuerza en las fibras musculares (42) o la depleción de glucógeno (43).

La fatiga que acontece tras un esfuerzo físico está condicionada por la magnitud de carga de una sesión (relación de volumen e intensidad). Debido a que la fatiga se considera la incapacidad del sistema neuromuscular de mantener un nivel requerido de potencia (44) y a que el rendimiento en un salto vertical con contramovimiento (CMJ) refleja las propiedades contráctiles y de control neuromuscular del aparato locomotor (45), la pérdida de rendimiento (altura de vuelo y/o potencia) durante la realización de un CMJ se ha considerado un indicador de fatiga mecánica y/o neuromuscular (46-48).

1.5 Metabolización del nitrato procedente del zumo de remolacha.

En la figura 5 vemos la ruta para la metabolización del nitrato inorgánico (NO_3^-) procedente del ZR; este es rico en NO_3^- y, en la cavidad oral, aproximadamente el 25% del NO_3^- procedente de la dieta se reduce a nitrito (NO_2^-) (49-50) , por acción de la enzima NO_3^- reductasa. El NO_2^-, por acción de los ácidos del estómago, es reducido parcialmente a óxido nítrico (NO) que, posteriormente, será absorbido a nivel intestinal (51) pasando al plasma sanguíneo. Además de esta vía para sintetizar NO, existen otras vías como en situaciones de hipoxia (52) , donde el metabolismo no depende de reacciones proveniente

de la oxidación; durante este estado se produce una disminución de la presión parcial de oxígeno y del pH (53) favoreciendo la reducción de NO_2^- a NO (54-55).

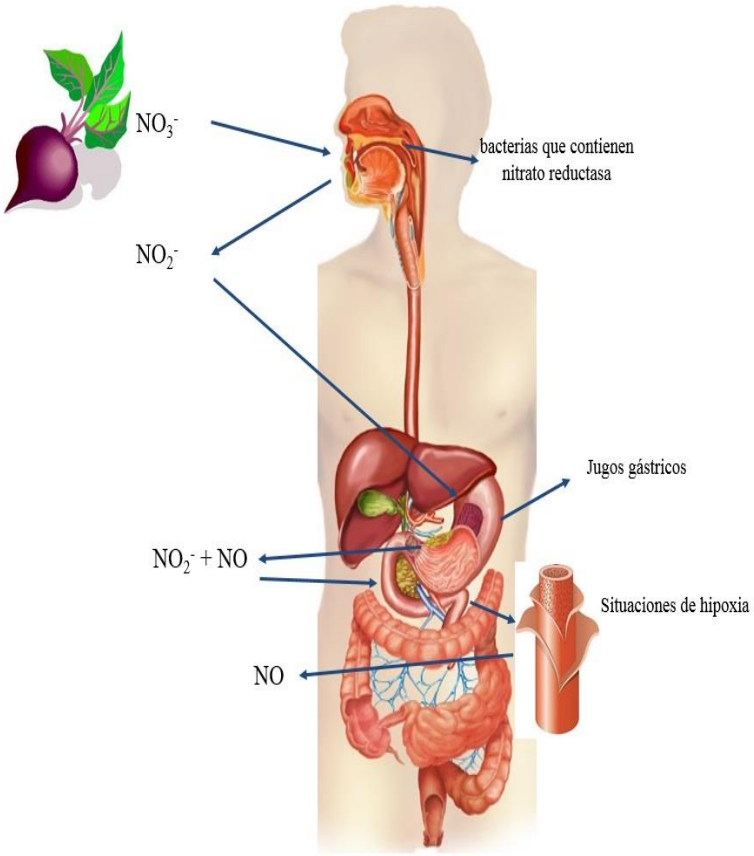

Figura 5. *Metabolización del nitrato procedente del zumo de remolacha*

1.5.1 Funciones del óxido nítrico.

El NO fue identificado como neurotransmisor parasimpático inhibitorio no adrenérgico, no colinérgico (56); este gas incoloro, al carecer de carga puede difundir por las membranas celulares de forma más rápida (57).

El NO presenta numerosas funciones fisiológicas, siendo destacables las relacionadas con mejoras hemodinámicas y metabólicas (54,58), dilatación vascular y aumento del flujo

sanguíneo muscular (59-60), favoreciendo los procesos de intercambio gaseoso en la fibra muscular (61). El NO es un estimulador de la expresión génica (62), aumentando la biogénesis (63) y eficiencia mitocondrial (64). Además de los efectos anteriormente descritos del NO, también encontramos otros efectos a considerar, como la mejora en la perfusión sanguínea y en la funcionalidad de las unidades motoras tipo II (60). Se ha comprobado, en las unidades motoras tipo II, mejoras en los procesos de salida y posterior recaptación de calcio desde el retículo sarcoplasmático (65), lo que se podría traducir en un aumento de la capacidad de producción de fuerza muscular por parte de las unidades motoras tipo II. La controversia generada sobre los posibles efectos fisiológicos positivos del ZR en deportistas entrenados, junto con la poca literatura existente sobre la suplementación con ZR en base a los distintos esfuerzos físicos de alta intensidad, así como algunos mecanismos fisiológicos que siguen sin estar claros, hacen que se tenga que profundizar en su estudio para ampliar más información sobre sus efectos en distintas modalidades deportivas.

1.5.2 Efectos del zumo de remolacha en el rendimiento aeróbico.

Encontramos varios artículos que hablan sobre la mejora en el rendimiento aeróbico en deportes de resistencia debido a la suplementación aguda con ZR. Kelly cols. (66) observaron en deportistas entrenados que el NO_3^- mejoraba el rendimiento ante intensidades al 60%, 70%, 80% y 100% del $VO_{2máx}$.Wilkerson y cols. (67) encontraron mejoras en un 0,8% en el tiempo total tras la realización de un test de 50 millas en ciclistas entrenados; Thompson y cols. (68) estudiaron durante una prueba submáxima de 40 minutos (20 minutos al 50% $VO_{2máx}$ seguidos de 20 minutos al 70% $VO_{2máx}$), continuando seguidamente al 90% $VO_{2máx}$ hasta el agotamiento, encontrando una mejora en la

economía entre un 7-11% en los vatios desarrollados por cada litro de VO_2, así como un 16% de mayor tiempo hasta el agotamiento al 90% $VO_{2máx}$; Lansley y cols. (69) observaron en ciclistas entrenados una mejora del tiempo empleado tras realizar una contrarreloj de 4km. En kayistas entrenados, Muggeridge y cols. (70) encontraron mejoras en la utilización de VO_2 cuando paleaban a intensidades relativas al 60% $VO_{2máx.}$; Murphy y cols.(71) vieron que en atletas, había mejoras de un 5% en el tiempo en recorrer una prueba de 5000 metros.

Como observamos la mayoría de las mejoras vienen en las pruebas de campo específicas del deporte practicado en resistencia, pero también encontramos artículos donde no había ninguna mejora. Macleod y cols. (72) investigaron en ciclistas entrenados los efectos de la suplementación de ZR en situaciones de normoxia. Para ello, evaluaron parámetros ventilatorios y la frecuencia cardiaca en un test de 15 minutos a la potencia aeróbica máxima y seguidamente 10 km, no encontrando ninguna mejora. Arnold y cols. (73), estudiaron en corredores entrenados la suplementación de 70 ml de ZR (7 mmol) en situación de hipoxia, donde realizaban un test incremental progresivo (a 4000 metros) y un test de 10 km (a 2500 metros), no mejorando la marca en ninguno de los test realizados. Boorsma y cols. (74), comprobaron la suplementación de 210 ml de ZR (6,5 mmol) durante un test de 19 minutos (7 min al 50% VO_2max + 7 min al 65% VO_2max + 5 min al 80% VO_2max) + test de 1500 m en atletas entrenados. No hallaron ninguna mejora en las variables cardioventilatorias ni mejoras en el rendimiento de los tests.

1.5.3 Efectos del zumo de remolacha en el rendimiento anaeróbico

A nivel energético en esfuerzos de alta intensidad, la suplementación con ZR reduce la utilización fosfocreatina (PCr) para la producción de fuerza (75). Dado que la PCr es la fuente energética preponderante en actividades de máxima intensidad hasta 6 segundos, siendo progresivamente menor la contribución energética tras este tiempo (49), es posible que la suplementación con ZR, a nivel metabólico, pueda mejorar la eficiencia energética de dicho sustrato. De este modo, un mayor tiempo en alcanzar la depleción de la PCr habría facilitado la obtención de mayores niveles de potencia durante más tiempo. Se han encontrado diferentes publicaciones donde se mejoraba el rendimiento. Aucouturiery cols. (76) vieron mejoras en deportistas recreacionales próximas al 20% en el número de repeticiones realizadas y en el trabajo total, durante la realización de un test de 15 segundos de pedaleo al 170% $VO_{2máx}$, seguido de 30 segundos de recuperación.

Muggeridge y cols.(70) observaron en kayistas que se incrementaba la potencia generada en las dos últimas series, ante un esfuerzo intermitente consistente en 5 series de 10 segundos, con 50 segundos de recuperación. Rimer y cols. (77), comprobaron el efecto de la suplementación aguda de ZR sobre el rendimiento en un test máximo de 3 segundos en un cicloergómetro isoinercial, así como en un test de 30 segundos a máxima intensidad en un cicloergómetro isocinético, observando mejoras de la cadencia de pedaleo durante el test de 3 segundos. Clifford y cols. (78) , tras la suplementación aguda de ZR, observaron mejoras en deportistas entrenados en la recuperación de la altura de vuelo en un CMJ, tras la realización de un protocolo de 20 series de 30 metros seguidos y 30 segundos de recuperación.

1.6 Planteamiento del problema

Tras investigar los diferentes efectos que existen en la suplementación de ZR de forma aguda en esfuerzos de aeróbicos y en esfuerzos de alta intensidad, encontramos mucha controversia sobre los estudios publicados. Queda mucho por esclarecer sobre los potenciales efectos beneficiosos del NO_3^- en modalidades deportivas de alta intensidad y en esfuerzos aeróbicos, especialmente con respecto a:

- Dosis aguda de ZR adecuada.
- Suplementación aguda versus suplementación crónica. Relacionado con la suplementación crónica que dosis y durante cuanto tiempo es adecuada la ingesta de ZR
- El efecto ergogénico en el rendimiento de sujetos altamente entrenados.
- El tipo de pruebas realizadas en condiciones de laboratorio para evaluar los efectos de la suplementación con ZR

1.6.1 Controversia de los efectos del zumo de remolacha en esfuerzos aeróbicos.

Las evidencias científicas determinan el efecto ergogénico del ZR en el rendimiento durante test de laboratorio y pruebas de campo (67-70,79) , sin embargo, también se encuentran artículos donde no se mejoraban ninguna de las variables estudiadas (35,58,73,74,80,81). Los potenciales efectos positivos del ZR en el rendimiento cardiorrespiratorio en pruebas de resistencia con deportistas muy entrenados generan una importante controversia entre los diferentes estudios.

Este debate se ve incrementado cuando se hace alusión a la dosis adecuada para obtener un efecto ergogénico mediante el ZR. Las existencias de diferentes estudios sobre la dosis correcta en esfuerzos de resistencia generan mucha polémica en determinar cual es la cantidad de NO_3^- ideal aportada por el ZR. Por un lado encontramos los artículos de Peeling y cols.(82) ; Thompson y cols. (68); Muggeridge y cols.(83) Muggeridge y cols.(70); Wilkerson y cols.(67) ;Lansley y cols. (69) que en deportistas de resistencia encontraban mejoras tras una suplementación de forma aguda con una menor cantidad de NO_3^- (menos de 6,5 mmol). Por otro lado vemos artículos de Wylie y cols. (76); Tan y cols. (77); Peeling y cols. (82); Kelly y cols. (66); en esfuerzos de resistencia cardiorrespiratoria donde las mejoras se producían con dosis agudas superiores (mayores de 8 mmol de NO_3^-)

Una declaración consensuada del Comité Olímpico Internacional manifiesta que existen mejoras en el rendimiento con dosis de 8 mmol y periodos de suplementación de NO_3^- más largos (> 3 días) en atletas altamente entrenados (84). Además de este organismo existen varios estudios que corroboran mejoras en el rendimiento con la suplementación crónica de ZR (65,85-88), aunque todavía no se han determinado las pautas de administración correctas.

Respecto a quien afecta más el uso del NO_3^-, Wilkerson y cols. (67), comentan que los deportistas entrenados necesitan una mayor dosis de NO_3^- que las personas no entrenadas para mejorar su rendimiento.

Otro aspecto relevante por considerar es el tipo de prueba utilizada para evaluar el impacto del NO_3^- en el rendimiento durante esfuerzos prolongados de larga duración, especialmente en condiciones de laboratorio. Los test de tiempo hasta la extenuación miden la capacidad para aguantar más que el rendimiento per se. Estos protocolos han sido criticados por su pobre validez ecológica y su poca aplicabilidad práctica en diferentes modalidades deportivas de resistencia (89,90). Otros estudios han realizado test para valorar el rendimiento mediante pruebas contrarreloj en las cuales se cubre una distancia dada en el menor tiempo posible (91,92).

Uno de los principales problemas de este tipo de pruebas en condiciones de laboratorio es que, debido a la distancia y la duración de las mismas, resulta complicado realizar pruebas de familiarización a la distancia criterio seleccionada o específica de una modalidad de resistencia. Este factor podría provocar una variabilidad importante en el ritmo a seguir y, consecuentemente, del rendimiento final cuando se compara el efecto placebo con la suplementación propiamente dicha, sobre todo en sujetos con menos experiencia no habituados a este tipo de valoraciones (93). Dada la complejidad para diseñar test específicos que simulen las condiciones reales de la distancia en esfuerzos prolongados, podría ser interesante analizar los efectos de la suplementación con ZR a una intensidad correspondiente a VT1, en el cual se valora el rendimiento aeróbico y la transición hacia el metabolismo anaeróbico (VT2). El uso de cargas submáximas a la intensidad de los VT parece ser el mejor predictor del rendimiento de resistencia en pruebas de ciclismo (93), además de estar altamente correlacionados con el ritmo de carrera en las pruebas de maratón y triatlón (94-96). Los VT no son utilizados para determinar el efecto ergogénico del ZR en el rendimiento aeróbico de esfuerzos prolongados. La importancia de conocer cada fase en el rendimiento nos sirve como

predictor de la marca, así como guía para la reestructuración de los entrenamientos, y poder conseguir el objetivo deportivo (97-98).

La evaluación del rendimiento tras la suplementación aguda con ZR en un test a carga constante a intensidad de VT1 y VT2 nos ayudaría a esclarecer los mecanismos fisiológicos cardiorrespiratorios, energéticos y de eficiencia mecánica que subyacen en el metabolismo prioritariamente aeróbico y, además, durante su transición hacia el metabolismo anaeróbico.

1.6.2 Carencia de conocimiento en esfuerzos de alta intensidad

Existe un potencial efecto ergogénico de la suplementación con ZR de forma aguda ante esfuerzos de alta intensidad con un metabolismo predominantemente no oxidativo (65), pero actualmente son pocos los trabajos que han valorado los efectos de la suplementación con ZR sobre esfuerzos de alta intensidad encontrando publicaciones donde si habían mejoras (76,77,99,100)) y en otros estudios ningún tipo de mejora (70,78) existiendo una tendencia hacia un efecto positivo en esfuerzos de alta intensidad.

Además de la controversia sobre la existencia de mejoras o no en el rendimiento en esfuerzos de alta intensidad, no queda claro la cantidad o duración (suplementación aguda o crónica) de la ingesta del ZR. Hay pocos estudios donde tras una ingesta aguda de ZR tengan un efecto ergogénico con una cantidad de NO_3^- (6,4 mmol) (99), siendo las necesidades para observar un efecto positivo, por encima de 10,9 mmol (76) o incluso 11,2 mmol (77). Respecto a la duración de la suplementación con ZR de forma crónica,

la mayoría de publicaciones alternan entre 6 dias (100) , 7 días (101), 5 días (102), y con dosis de 8,4 mmol, 6,4 mmol y 12,8 mmol de NO_3^-, siendo menos estudios que los concernientes a la suplementación aguda.

Volvemos a encontrarnos diferentes resultados y, por tanto, controversias en la literatura, haciendo necesario profundizar en el uso de la suplementación de ZR en esfuerzos de alta intensidad. Estas discrepancias, tanto en esfuerzos aeróbicos y esfuerzos de alta intensidad, hacen necesario profundizar y realizar intervenciones experimentales para poder deducir cuales son realmente los beneficios de la suplementación de ZR, pautas respecto a las cantidades, tiempo en tomarlas, deportes donde más beneficio obtenemos ó fatiga muscular.

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2. OBJETIVOS E HIPOTESIS

2.1 Objetivo General.

De forma general, el principal objetivo de esta tesis doctoral es comprobar los efectos de la suplementación aguda con ZR en el rendimiento de esfuerzos prioritariamente anaeróbicos de alta intensidad y en el rendimiento cardiorrespiratorio de esfuerzos mayoritariamente aeróbicos prolongados, en diferentes deportistas.

2.2 Objetivos específicos

Tras las revisiones efectuadas sobre el efecto de la suplementación de ZR en deportes con un metabolismo mayoritariamente aeróbico (103) y en esfuerzos anaeróbicos de alta intensidad, se observó mucha controversia y pocos estudios en deportistas entrenados, por esta razón nos planteamos los siguientes objetivos específicos:

- Analizar los efectos del ZR en el rendimiento en triatletas entrenados, en una prueba constante a intensidades de VT1 y VT2 (artículo I).
    - Comparar las respuestas cardiorrespiratorias y metabólicas entre ambas condiciones experimentales (ZR vs PL)
    - Evaluar el comportamiento del componente lento del VO_2 comparando las condiciones experimentales (ZR vs PL)
    - Estudiar la eficiencia mecánica y la economía en ambas condiciones experimentales (ZR vs PL)
    - Investigar el gasto energético comparando ambas condiciones experimentales
- Determinar el estado actual de la literatura científica sobre la suplementación de ZR en deportes de alta intensidad (artículo II).

- Evaluar los efectos de la suplementación aguda con ZR en esfuerzos de alta intensidad anaeróbicos en un test de Wingate, en deportistas entrenados (artículo III y IV).
    - Examinar las concentraciones de lactato en sangre comparando ambas condiciones experimentales (ZR vs PL)
    - Estudiar el comportamiento de la potencia durante un test de Wingate comparando ambas condiciones experimentales (ZR vs PL)
- Estudiar los efectos de la suplementación aguda con ZR sobre la fatiga mecánica en un test de Wingate en deportistas entrenados, medida mediante un test de salto con contramovimiento (artículo IV).
- Establecer las pautas y cantidades correctas para la toma aguda de este suplemento durante la realización de esfuerzos de alta intensidad anaeróbicos y esfuerzos prolongados aeróbicos (artículos I,III, IV).

2.3 Hipótesis.

Dada la diferente naturaleza metabólica de los estudios planteados en esta tesis doctoral, se han establecido dos hipótesis de trabajo:

1- La suplementación aguda con ZR mejora el rendimiento cardiorrespiratorio y metabólico, el gasto energético y la eficiencia mecánica en un test a carga constante a intensidades de VT1 y VT2, en triatletas altamente entrenados.

2- La suplementación aguda con ZR permite incrementar el rendimiento mecánico (potencia) y metabólico, disminuyendo la fatiga mecánica en un test de Wingate.

3. INFORME DEL DIRECTOR SOBRE LOS ARTÍCULOS CIENTÍFICOS PUBLICADOS

El Dr. Manuel Vicente Garnacho Castaño declara, como director de la tesis doctoral presentada por el doctorando Eduardo Cuenca García titulada "Efecto de la suplementación aguda con zumo de remolacha en el rendimiento aeróbico y anaeróbico", la autenticidad del factor de impacto de los artículos *Journal Citation Reports* presentados, así como su implicación y dedicación en la elaboración de dichos artículos científicos que conforman esta tesis doctoral.

Eduardo ha participado de forma proactiva en la elaboración de los artículos, como queda reflejada en la relación de autores de todos los artículos, figurando como primer autor en uno de ellos.

Concretamente en el primer artículo, como queda reflejado en el artículo original, el doctorando realizó toda la fase experimental, contribuyó a la preparación de todos los materiales y herramientas de análisis, así como aprobó la versión final del manuscrito. (Author's contribution, pag. 11). En el segundo artículo de revisión, el doctorando seleccionó y analizó el contenido de los artículos incluidos en el manuscrito, preparó las figuras, tablas y redactó el manuscrito, editó y revisó el manuscrito, así como aprobó su versión final (Author's contribution, pag. 9). En el tercer artículo el doctorando realizó toda la fase experimental y contribuyó a la escritura de la versión final del manuscrito (Author's contribution, pag. 11).En el cuarto artículo, además de realizar la fase experimental, analizó los datos y escribió el manuscrito (Author's contribution, pag. 9).

A continuación, se detalla la relación de artículos que conforman esta tesis:

Artículo I:

Título: Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts.

Autores: Domínguez R, Maté-Muñoz JL, **Cuenca E**, García-Fernández P, Mata Ordoñez F, Lozano-Estevan MC, Veiga-Herreros P, Fernandes da Silva S, Garnacho-Castaño MV**.** Revista: Journal of the International Society of Sports Nutrition.

DOI: DOI 10.1186/s12970-017-0204-9. Año: 2018

ISSN: 1550-2783; Factor de Impacto (2018): 3,841, Cuartil: Q1

Artículo II:

Título: Effects of a single dose of beetroot juice on cycling time trial performance at ventilatory thresholds intensity in male triathletes.

Autores: Garnacho-Castaño MV, Palau-Salvà G, **Cuenca-García E**, Muñoz-González A, García-Fernández P, Lozano-Estevan MC, Veiga-Herreros P, Maté-Muñoz JL, Domínguez R.

Revista: Journal of the International Society of Sports Nutrition

DOI: doi.org/10.1186/s12970-018-0255-6; Año: 2018

ISSN: 1550-2783; Factor de Impacto (2018): 3,841, Cuartil: Q1

Artículo III:

Título: Effects of beetroot juice supplementation on a 30-second high intensity effort.

Autores: Domínguez R, Garnacho-Castaño MV, **Cuenca E**, García-Fernández P, Muñoz-González A, de Jesús F, Lozano-Estevan MC, Fernandes da Silva S, Veiga-Herreros P, Maté-Muñoz JL**.**

Revista: Nutrients.

DOI: doi:10.3390/nu9121360, Año: 2017

ISSN: ISSN 2072-6643; Factor de Impacto (2017): 4,196, Cuartil: Q1

Artículo IV:

Título: Effects of beetroot juice supplementation on performance and fatigue in a 30-s all-out sprint exercise: A randomized, double-blind cross-over study.

Autores: **Cuenca E**, Jodra P, Pérez-López A, González-Rodríguez L, Fernandes da Silva S, Veiga-Herreros P, & Domínguez R.

Revista: Nutrients.

DOI: doi:10.3390/nu10091222, Año: 2018

ISSN: ISSN 2072-6643; Factor de Impacto (2017): 4,196, Cuartil: Q1

Sinceramente,

Dr. Manuel V. Garnacho Castaño

En Barcelona, a 2 de septiembre de 2019

4. ARTÍCULOS CIENTIFICOS

En la presente tesis doctoral se presentan 4 Journal Citation Reports, de los cuales un artículo es una revisión sistemática sobre esfuerzos de alta intensidad y tres artículos son intervenciones experimentales. En las intervenciones experimentales un artículo versa sobre esfuerzos de resistencia prioritariamente aeróbicos prolongados y otros dos sobre esfuerzos de alta intensidad en un metabolismo mayoritariamente anaeróbico.

ARTÍCULO I.

EFFECTS OF BEETROOT JUICE SUPPLEMENTATION ON INTERMITTENT HIGH-INTENSITY EXERCISE EFFORTS

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ABSTRACT

Beetroot juice contains high levels of inorganic nitrate (NO_3^-) and its intake has proved effective at increasing bloodnitric oxide (NO) concentrations. Given the effects of NO in promoting vasodilation andblood flow with beneficial impacts on muscle contraction, several studies have detected an ergogenic effect of beetroot juice supplementation on exercise efforts with high oxidative energy metabolism demands. However, only a scarce yet growing number of investigations have sought to assess the effects of this supplement on performance at high-intensity exercise. Here we review the few studies that have addressed this issue.

The databases Dialnet, Elsevier, Medline, Pubmed and Web of Science were searched for articles in English, Portuguese and Spanish published from 2010 to March 31 to 2017 using the keywords: beet or beetroot or nitrate or nitrite and supplement or supplementation or nutrition or “sport nutrition” and exercise or sport or “physical activity” or effort or athlete. Nine articles fulfilling the inclusion criteria were identified. Results indicate that beetroot juice given as a single dose or over a few days may improve performance at intermittent, high-intensity efforts with short rest periods. The improvements observed were attributed to faster phosphor creatine resynthesis which could delay its depletion during repetitiveexercise efforts. In addition, beetroot juice supplementation could improve muscle power output via a mechanism involving a faster muscle shortening velocity. The findings of some studies also suggested improvedindicators ofmuscular fatigue, though the mechanism involved in this effect remains unclear.

KEYWORDS

Beet, ergogenic aids, exercise, sport supplement

INTRODUCTION

Because of the increase in competitive equality in high level sport, a 0.6% performance improvement is today considered sufficient to make a difference[1]. In this setting of high competition, athletes often look to nutritional supplements to boost their performance [2]. However, most statements about the potential effects on sport performance or health that appear on the labels of many products are not backed by clear scientific evidence [2]. Because of this, institutions such as the Australian Institute of Sport(AIS) have created a system to classify supplements according to their effects on performance based on confirmed scientific evidence[3]. Thus, dietary supplements assigned to class A have been proven with a high level of evidence to improve exercise performance in certain modalities when taken in appropriate amounts. The only substances in this class are β-alanine, sodium bicarbonate, caffeine, creatine and beetroot juice [4]. However, it is thought that the effect of a given supplement on performance besides the recommended dose may be specific to each sport's modality [5]. This, in turn, will depend on the energy and/or mechanical requirements of each form of exercise such that some supplements will have an ergogenic effect on some types of exercise efforts and have no effects on other types.

The relationship between exercise intensity and time to exhaustion is hyperbolic [6]as it is directly linked to the prevailing energy producing systems during exercise[7]. Thus, depending on their bioenergetics, the different exercise efforts can be classified according to exercise duration. This means we can differentiate between explosive efforts, high-intensity efforts and endurance-intensive efforts[8].Explosive efforts are those lasting under 6 seconds in which the main energy metabolism pathway is the high-energy phosphagen system and there is some participation also of glycolysis [9,10], which gradually contributes more energy until 50% at 6 seconds[9]. High-intensity efforts are those of duration longer than 6 seconds and shorter than 1 minute[11]. These efforts are characterized by a major contribution of glycolytic metabolism and smaller contribution of high-energy phosphagens and oxidative phosphorylation[8]. Finally, intensive endurance efforts are those lasting longer than 60 seconds and whose main energy producing system is oxidative phosphorylation[8].

Beetroot juice is used as a supplement because it may serve as a precursor of nitric oxide (NO) [12].The mechanism of NO synthesis is thought to be via the catabolism of arginine by the enzyme NO synthase [13]. Effectively, arginine supplementation has been shown to increase NO levels[14]. An alternative mechanism of NO genesis is mediated by inorganic nitrate (NO_3^-). This means that the high amounts of NO_3^- present in beetroot juice are able to increase NO levels in the organism.

In the mouth, some 25% ofdietary $NO_3^-$is reduced byNO_3^- reductase produced by microorganisms[15] to nitrite (NO_2^-)[16]. This $NO_2^-$is then partially reduced to NO through the actions of stomach acids which is later absorbed in the gut[17].Some of this NO_2^- enters the bloodstream, and,in conditions of low oxygen levels, will be converted into NO [18] (Figure 1).

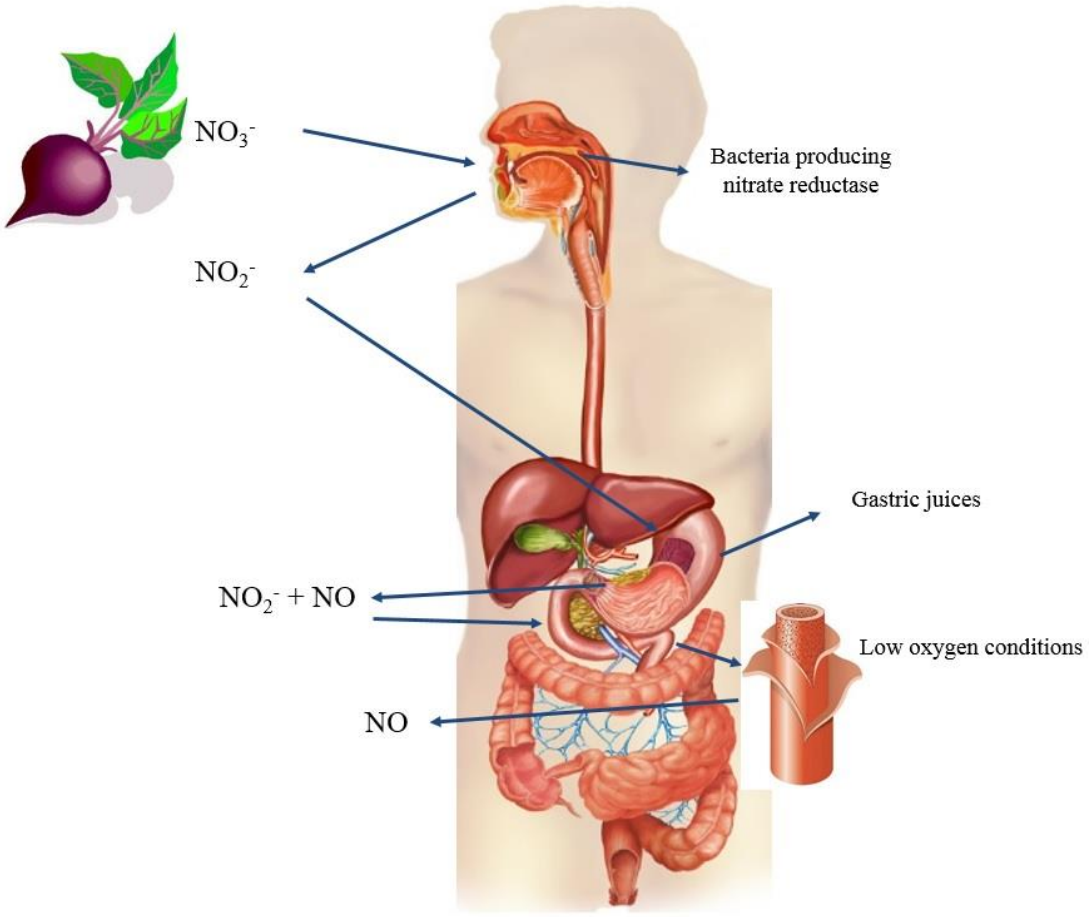

Figure 1. Conversion of NO_3^-in beetroot juice to NO. The diagram shows how ingested $NO_3^-$is transformed by bacteria in the mouth containing nitrite reductase

to NO_2^-. Once in the gut, NO_2^-enters the bloodstream and, under conditions of hypoxia,is used to generate NO.

Nitrous oxide has numerous physiological functions including hemodynamic and metabolic actions[19,20]. Mediated by guanyl cyclase [21], NO has an effect on smooth muscle fibres causing blood vessel dilation [22]. This vasodilation effect increases blood flow to muscle fibres[23] promoting gas exchange[24]. NO also induces gene expression[25],enhancing biogenesis [26] and mitochondrial efficiency [27]. All these effects can favour an oxidative energy metabolism. In effect, though not all[28-31],numerous investigations have noted that beetroot juice supplementation boosts performance in exercise modalities involving intensive endurance efforts in which the dominant type of energy metabolism is oxidative [24,27,32-45].

To date, several reviews of the literature have assessed the effects of beetroot juice supplements on physical exercise [12,46-49]. In addition, given that NO can potentiate the factors that limit performance when executing actions in which the predominant metabolism is oxidative, two recent reviews have explored the positive effects of this form of supplementation on endurance exercise [50-51]. Thus, the different studies showed that beetroot juice supplementation was effective at: lowering VO_2 by-6%during a swimming test conducted at an intensity equivalent to the ventilatory threshold (VT) [27]; lowering VO_2 by-3% during a kayaking test conducted at 60% VO_{2max} [38] and during a cycle ergometry test conducted by recreation sport athletes [45] and cyclists [34]at 45-70% VO_{2max}; increasing performance by 12-17% in cycle ergometry tests until exhaustion conducted at intensities of 60 to 90% $VO_{2max}$by recreation sport athletes [37, 42], and by 22% when conducted at a 70% intensity between VT and VO_{2max}[36]; and finally, improving times by 2.8% in trained cyclists conducting cycle ergometery tests of 4 km [33], 10 km (1.2%)[34], 16 km (2.7%)[33] and 50 miles (0.8%)[35].

However, besides the effects of NO mentioned above, other impacts need to be considered. Accordingly, it has been described that the effect of increased blood flow induced by NO is specific totype II muscle fibres [20]. Moreover, in type II muscle fibres, beetroot juice intake has been found to improve the release and later reuptake of calcium from the sarcoplasmic reticulum [52]. This could translate to an increased capacity for muscle strength production of these type II

muscle fibres.Such effects of NO could mean a physiological advantage for efforts involving the recruitment of type II muscle fibres, such as intermittent, high-intensity efforts. Hence, given the scarce yet growing number of studies that have addressed the effects of beetroot juice supplementation on this type of intermittent, high-intensity effort [38,53-60],here we review the results of experimental studies that have specifically examined in adults (whether athletes or not) the effects of beetroot juice supplementation on intermittent, high-intensity efforts.

METHODOLOGY

We identified all studies that have assessed the effects of BJ supplementation on intermittent, high-intensity efforts by searching the databases Dialnet, Elsevier, Medline, Pubmedand Web of Science published up untilMarch 31, 2017 using the keywords:beet OR beetroot OR nitrate OR nitrite(concept 1) AND supplement OR supplementation OR nutrition OR “sport nutrition” (concept 2) AND exercise OR sport OR “physical activity” OR effort OR athlete (concept 3).

Two of the present authors (E.C and P.G-F) first eliminated duplicate articles and then removed descriptions of studies that were not experimental, were not written in English or Spanish, or were published before 2010. This meant that all the studies reviewed were published over the period January 1, 2010 to March 31, 2017. Next, these two same authors applied a set of exclusion criteria to ensure the selection only of studies specifically designed to assess the effects of BJ supplementation on intermittent, high-intensity efforts:

- Studies performed in non-adults (samples including subjects aged <18 or>65 years).
- Studies conducted in vitro or in animals.
- Studies in which the direct effects of BJ were not determined.
- Studies in which impacts were examined on exercises that did not comply with the characteristics of intermittent, high-intensity efforts.

If there was disagreement about whether a given study met the inclusion/exclusion criteria, the opinion of a third researcher (F.M-O) was sought.

RESULTS

Study selection

Of 738 studies identified in the search, 359 were left after eliminating repeated records. Once, the titles and abstract of these 359 publications were reviewed, 212 full text articles were indentified and retrieved for assessment,of which 9 articles met the elegibility criteria (Figure 2).

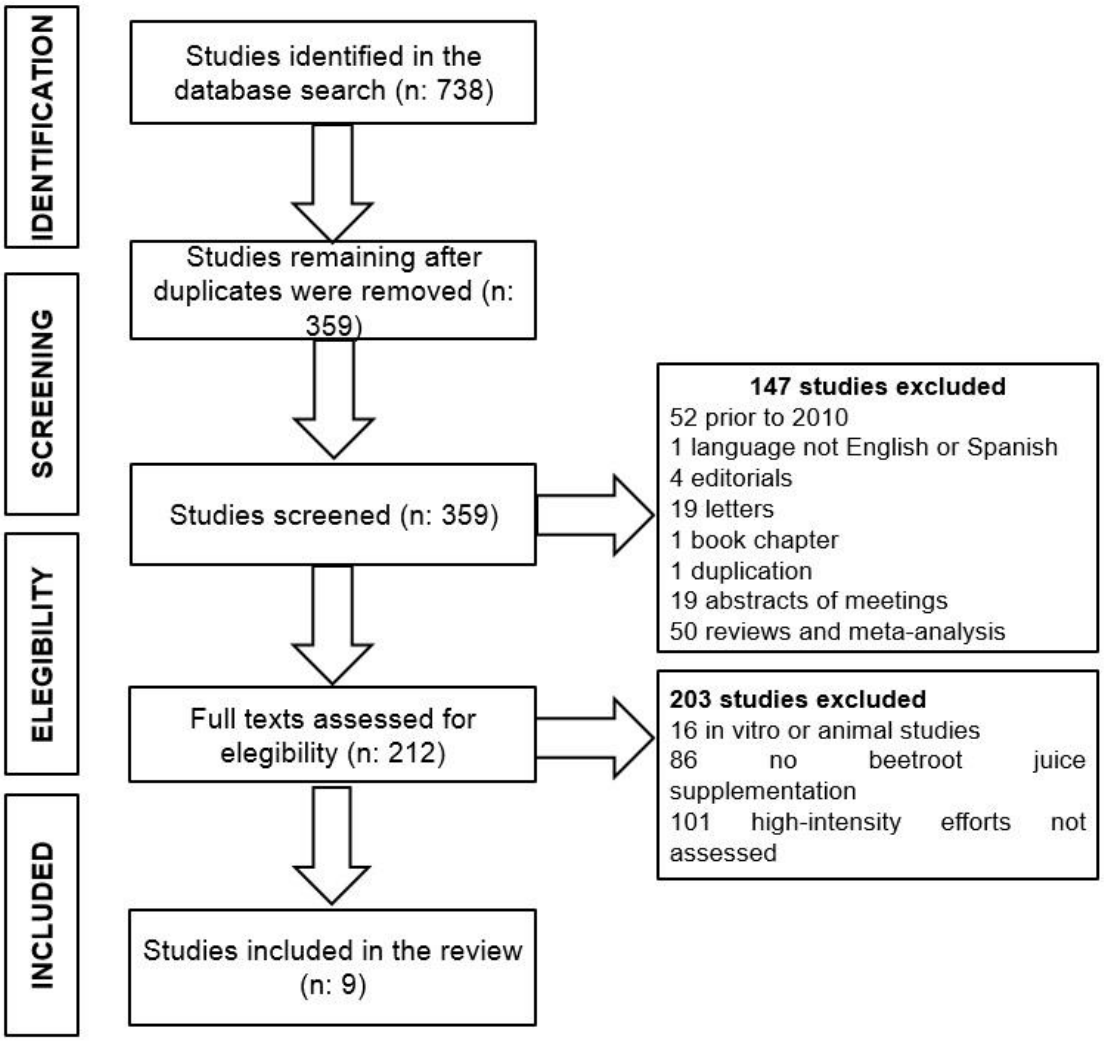

Figure 2. Article selection

Study characteristics

The nine studies selected for our review included a total of 120 subjects, 107 of whom were men and 13 women.

In five of these studies [38,53,54,57,59], the effects of a single beetroot juice supplement (acute effects)were assessed. The supplement was taken 120 min before exercise in one study [53], 150 min before exercise in two [57,59] and 180 min before exercise in the remaining two[38,54].

In the remaining four studies, the effects of chronic beetroot juice supplementation were examined [55,56,58,60]. The supplementation periods were 5 days in one study[60],6 days in two [55,58] and seven days in the fourth study[56].

Doses of NO_3^- ingested ranged from ~5 mmol [38]to ~11.4 mmol [57]. In addition, one study examined the efficacy of beetroot juice taken separately or in combination with sodium phosphate [55].

In four of the nine studies reviewed, participants were competition athletes [38,55,57,59] and in the other five they were recreation sport or low-level competition athletes [53,54,56,58,60]. Only one of the study populations included athletes of individual sports modalities[38],the rest of the studies were conducted in players of team sports [53-60].

The tests used to assess performance were a 30-s duration cycle ergometer test in one [59]and high-intensity, intermittent exercises in the remaining studies with work intervals ranging from 6 s [58]to 60 s [60]and rest periods from 14 s [56]to 4 min [60]. The types of tests employed were running at maximum speed in three studies[55-57], cycle ergometry in four[53,54,59,60], one of which was an isokinetic test [59],a kayak ergometer test in one [38]and bench press strength training in the remaining study[58].

The beetroot juice intervention led to significantly improved performance in four of the studies[54,56,58,60],while in another four no such effects were observed[38,55,57,59]. In the remaining study, an ergolytic, or reduced performance, effect was noted in relation to the placebo treatment.

Study results

In Table 1 we summarize the results of the nine studies reviewed and provide details on the participants, experimental conditions, supplement regimens, and performance tests employed.

Reference	Subjects	Study design	Dose	Exercise test	Results
Muggeridge et al. [38]	Trained kayakers (male, n=8) (VO_{2peak}49.0±6.1 ml·kg·min^{-1})	Single-blind, randomized, cross-over	5 mmol NO_3^- (180 min before)	Kayak ergometer: 5 x 10 s sprint-rest 50 s	+4% average power(420±23 vs 404±24 W)
Martin et al. [53]	Recreation team sport players (male, n=16) (VO_{2peak}47.2±8.5 ml·kg·min^{-1})	Double-blind, randomized, cross-over	6.4 mmol NO_3^- (120 min before)	Cycle ergometer: sets until exhaustion of 8 s–rest 30 s	-13% reps (13±5 vs 15±6)and -17% total work(49.2±24.2 vs 57.8±34.0kJ)
Aucouturier et al. [54]	Recreation team sport players (male, n=12) (VO_{2peak}46.6±3.4 ml·kg·min^{-1})	Single-blind, randomized, cross-over	10.9 mmol NO_3^- (180 min before)	Cycle ergometer: sets until exhaustion of 15 s at 170% MAP–rest 30 s	+20% reps* (26.1±10.7 vs 21.8±8.0)and 18% total workload*(168.2±60.2 vs 142.0±46.8kJ)
Buck et al. [55]	Amateur team sport players (female, n=13) (VO_{2peak} notspecified)	Double-blind, randomized,Latin-square	BJ: 6.4 mmol NO_3^- (6 days) BJ+SP: 6.4 mmol NO_3^- + 50 mg·kg lean mass SP (6 days)	PRE, MID and POST simulation team sport matches: 6x(20 m sprint+rest25 s)	BJ: -0.2% total sprint time per set(69.8±4.9 vs 69.97±4.2) BJ+SP: -2% total sprint time perset(68.9±5.1 vs 69.97±4.2)

Thompson et al. [56]	Recreation team sport players (male, n=16) (VO_{2peak}50±7 ml·kg·min^{-1})	Double-blind, randomized, cross-over	12.8 mmol NO_3^- (7 days)	MID and POST simulated team-sport matches: 2x[5x(6 s cycle ergometrysprint+rest14 s)]	5% work volume at MID*(63±20 vs 60±18kJ), 2% POST(60±17 vs 59±16kJ) and 4% whole session*(123±19 vs 119±17kJ)
Clifford et al. [57]	Competition team sport players (male, n=20) (VO_{2peak} not specified)	Double-blind, independent groups design	11.4 mmol NO_3^- (150 min before)	2xRST: 20x(30 m sprint–rest 30 s)	-1% average sprint time RST1(4.65±0.3 vs 4.7±0.2 s)and -2% RST2(4.66±0.2 vs 4.77±0.2 s)and -2% fastest sprint RST1 (4.41±0.2 vs 4.48±0.1 s)and -3%RST2(4.38±0.2 vs 4.53±0.2 s)
Mosher et al. [58]	Recreation sport players (male, n=12)(VO_{2peak} not specified)	Double-blind, randomized, cross-over	6.4 mmol NO_3^- (6 days)	Bench press: 3x(maximum number reps at 60% 1 RM)	+ 19% weight lifted in sessionand improved no. of reps S1*, S2*, S3* and whole session. *improvements not specified
Rimer et al. [59]	Competition sport players (male, n=13)(VO_{2peak} not specified)	Double-blind, randomized, cross-over	11.2 mmol NO_3^- (150 min before)	Isokinetic cycle ergometer: Wingate 30-s test	-1% peak power(1173±255 vs 1185±249 W) and -1% total work(22.8±4.8 vs 23±4.8 W)
Wylie et al. [60]	Recreation team sport players (male, n=10) (VO_{2peak}58±8 ml·kg·min^{-1})	Double-blind, randomized, cross-over design	8.4 mmol NO_3^- (5 days)	Cycle ergometer : 24 x(6 s sprint–rest 24 s) Cycle ergometer : 7 x (30 s sprint–rest 4 min) Cycle ergometer : 6 x (60 s sprint–rest 60 s)	+5% mean averagepower*(568±136 vs 539±136W)and +1% mean peak power(792±159 vs 782±154 W) in 24 x (6 s sprint–rest 24 s); -1% mean average power (558±95 vs 562±94 W)and -1% mean peak power (768±157 vs 776±142W)in 7 x(30 s sprint–rest 4 min)

BJ: beetroot juice; MID: half-time simulation match; n: sample size; no.: number; NO_3^-: nitrate concentration in the drink; MAP: maximum aerobic power; POST: end simulation match; PRE: before simulation match; Rep: repetition; RST: repeated sprint test; SP: sodium phosphate; VO_{2peak}: peak oxygen consumption; *: statistically significant differences.

DISCUSSION

Effects of chronic supplementation with beetroot juice on intermittent, high-intensity exercise efforts

Four of the studies reviewed tested the effects of taking beetroot juice supplements for 5 to 7 days on intermittent, high-intensity efforts[55,56,60] or on a resistance training session[58].Three of these studies detected a significant effect of beetroot juice supplementation [56,58,60] while in the remaining study, no significant difference compared with the placebo was noted [55].

Effects of chronic supplementation with beetroot juice onresistance training

Resistance training is used to improve muscular hypertrophy, strength, power and muscular endurance[61]. Training sessions targeting muscle hypertrophy include workloads of around 70-85% 1 RM and 8-12 repetitions, while those aiming to improve muscular endurance include loads of around 50% 1 RM and some 15-25 repetitions[62]. Such exercise sessions are largely dependent on glycolytic metabolism; the lactate threshold in resistance training exercises such as half squat is detected at ~25% 1 RM [63,64]. To determine the effects of 6 days of beetroot juice supplementation (6.4 mmol NO_3) on resistance training sessions designed to improve local muscular hypertrophy and endurance, in the study by Mosher et al. reviewed here [58],the number of bench press repetitions accomplished in three sets

using loads equivalent to 60% 1 RM was recorded. Results indicated that supplementation increased the number of repetitions in the three exercise sets improving session performance by 18.9%.

In an earlier investigation, the effects of sodium bicarbonate supplements were assessed in a similar study to the one by Mosher et al. [58]. Subjects performed 3 sets until exhaustion with loads of 10-12 RM in three exercises targeting the lower limbs [65]. Results indicated that, like the beetroot juice, sodium bicarbonate supplementation led to more repetitions in the session [65]. However, in parallel with the increasing number of repetitions, blood lactate concentrations also rose (~2.5 mmol) [65]. This was not observed in Mosher's study [58].

If we consider the nature of resistance training, the athlete passes from a resting condition to a situation demanding high energy levels during the first repetitions of a set. Because the phosphagen system is the main energy pathway in rest-exercise transitions [66], phosphocreatine reserves may be depleted in response to a resistance training exercise set. Recovering these reserves takes some 3-5 minutes [67]. Given that phosphocreatine resynthesis is dependent on oxidative metabolism [68]and that beetroot juice has an ergogenic effect on exercise modalities with a major oxidative metabolism component [50], it could be that this supplement accelerated this recovery during the rest period in Mosher's study(2 minutes) and thus avoided progressive phosphocreatine depletion throughout the session.

In turn, this faster rate of resynthesis would attenuate the increasing levels of adenosine diphosphate (ADP) and inorganic phosphates [68]. Both these metabolites have been associated with the appearance of muscular fatigue [70]. Hence, by delaying the build-up of critical levels of these metabolites, the appearance of fatigue will be delayed and this will allow for more repetitions in sets until exhaustion [58]. NO_3^- supplementation could also improve muscle efficiency

and contractile capacity by promoting the release of calcium from the sarcoplasmic reticulum in the muscle cells and its reuptake[52,70]. Thus, a train of action potentials leading to an increased supply of calcium to the muscle fibre will increase the strength of muscle contraction[13].

Effects of chronic supplementation with beetroot juice on intermittent high-intensity exercise efforts

Some sport modalities such as team, racket or combat sports require bursts of high-intensity efforts followed by rest periods. Thus, in team sports, high-intensity efforts (~3-4 s) are interspersed with variable active rest periods [72]. In racket sports like tennis, efforts last 7-10 s and rest periods 10-16 s (between points)and/or 60-90 s(side changes) [73]. Finally, in combat sports more intense efforts are 15-30 s long and active rest periods are 5-10 s long every 5 min[74]. In all these sports modalities, the capacity to repeat high-intensity efforts with only short recovery periods is considered a performance indicator [75]. This means that higher level athletes are able to maintain performance in successive high-intensity intervals over a long time period [76].

To find out if beetroot juice supplementation would improve this ability to repeat high-intensity efforts during a team sport match, Thompson et al.[56]administered beetroot juice over 7 days to a group of athletes (12.8 mmol NO_3^-). The performance test consisted of two blocks of five 6-s sets of sprints on a cycle ergometer with 14-s active recovery periods in the middle and end of a simulated match lasting 2 x 40 min [56]. The results of this study indicated a total work volume improved by 3.5% in the whole session, though this improvement was greater at the end of the first half (at half time).

If we again consider the nature of this type of exercise, it has been established that it involves the recruitment of type II muscle fibres[77,78], which are more

powerful though show more fatigue than type I units[79]. This lesser resistance to fatigue has been related to reduced blood flow and myoglobin concentrations in these muscle fibres compared to type I. Hence, type II muscle fibres are designed to promote non oxidative pathways and have shown a greater creatine storage capacity [80] for an enhanced metabolism of phosphocreatine [81]and proteins with a buffering effect at the intracellular level such as carnosine [82], favouring a glycolytic type metabolism.

Animal studies have shown that increased blood flowin response to NO_3^- supplementation is greater in type II compared to type I muscle fibres[20]. This greater irrigation and oxygen availability in the recovery period along with a greater creatine storage capacity of motor type II units[80](promoting phosphocreatine resynthesis[81]) means that during an exercise effort followed by a short rest period (14 s), beetroot juice supplementation could delay phosphocreatine depletion during successive sprints and explain the improvements noted by Thompson et al. [56].

Despite such greater effects of NO_3^-supplementation on type II versus type I muscle fibres, animal studies have also shown that effects on calcium release and reuptake in the muscle cell sarcoplasmic reticulum is greater in type II than type I muscle fibres[52]. Accordingly, because of the important role of type II muscle fibres during sprints [77,78], supplementation could have led to an improved capacity to generate muscle power and thus explain the significant improvements in performance observed by Thompson's group.

Buck et al. [55] examined the effects of 6 days of supplementation with beetroot juice (6.4 mmol NO_3^-) or sodium phosphate (50 mg·kg lean mass)on performance in a test consisting of repeated sprints as 6 sets of 20 m and 25-s of rest between sets in the middle and end of a simulated match lasting 60 min. The beetroot juice intervention did not improve performance at these sprints, yet did do so when taken along with sodium phosphate (2%) compared with placebo, though

this improvement was of lesser magnitude than when the subjects only took sodium phosphate supplements (5%). These findings suggest that, unlike beetroot juice, sodium phosphate intake may have an ergogenic effect in this protocol. If we compare the tests used by Buck et al. [55]and Thompson et al. [56], work periods were shorter (2-3vs 6 s), while rest periods were longer (25 vs 14 s). Therefore it could be that 2-3 s efforts lead to a significantly lower reduction of phosphocreatine reserves at the end of these efforts. Further, the 25 s of rest approaching the 30 seconds in which the recovery of 50% of phosphocreatine stores takes place [67], may have been sufficient to stabilize reserves of phosphocreatine and therefore avoid the appearance of fatigue [83].

Another study investigated the effects of longer term supplementation (5 days) with beetroot juice (8.4 mmol NO_3^-), this time on performance in a repeated high-intensity test[60]. These authors sought to determine supplementation effects on different exercise protocols. Subjects performed a session consisting of twenty four 6-s sets of work and 24 s of rest between sets, a second session of two 30-s sets of work and 2 min of rest between sets and a third session of six 6-s sets and 60 s of rest between sets. As did Thompson et al. [56], Wylie et al. [60]selected 6-s exercise sets in the first session though rest intervals were longer (24 vs 14 s). Another difference was that the participants had not first undergone fatigue (in the simulated team sport match) before the performance test. Notwithstanding, results were similar in that mean power generated in the sets over a whole session improved by ~7%. However, improvements across the 24 x 6-24 protocol were not comparable to those recorded in the other two tests, in which no significant improvements were recorded.

In the test protocols including 30-sand 60-s work efforts, beetroot juice supplementation resulted in no improvements in any indicators of performance [60]. These protocols consisting of longer duration work intervals mainly involve a glycolytic type metabolism and in smaller measure elicit the high-energy

phosphagen system. An increase in glycolysis leads to increased H^+ production, lowering pH [84]. To avoid increasing acidosis, a series of responses targeted at reducing phosphor fructokinase take place including diminished glycolysis [85] and phosphocreatine resynthesis[86], and muscle contractibility modifications [87]. Such responses manifest as reduced non aerobic metabolism or a reduced capacity for muscle power and strength, in other words, fatigue [88].Supplements such as β-alanine (which increases muscle carnosine concentrations [89], a protein that acts as a buffer inside the cell [90]) and sodium bicarbonate[91] (main extracellular buffering agent) have shown ergogenic effects on performance at high-intensity efforts involving the predominance of glycolytic metabolism[92]. The combined effect of these supplements is greater than the impact of each supplement on its own[93].

Although beetroot juice supplementation induces vasodilation and increased blood flow (in type II muscle fibres, recruited mainly in exercise bouts of 30 to 60 s duration), increasing available oxygen in the muscles, rather than being activated because of a lack of oxygen (anaerobiosis), non-oxygen dependent pathways are activated because of a greater demand for energy production via oxidative phosphorylation. Thus, these effects, although they potentiate oxidative phosphorylation, have no repercussions on glycolytic energy metabolism. Hence, as beetroot juice has no alkalizing effect supplementation with this product is unable to reduce acidosis, as the main factor limiting performance at efforts lasting 30-60 s. However, potentiating effects on aerobic metabolism increases the speed of phosphocreatine resynthesis, dependent on oxidative phosphorylation. This means it may be effective for repeated high-intensity efforts whose duration is close to 6-10 s, in which high energy phosphagens contribute mainly to the metabolism [94]and the work volume is sufficient to cause significant depletion, which when faced with short rest intervals leads to progressive depletion and consequently to fatigue. Accordingly, beetroot juice supplements can have an ergogenic effect when exercise

efforts are intermittent, maximum intensity, short-duration (6-10 s) and interspersed with brief recovery periods(<30 s).

Effects of acute beetroot juice supplementation on intermittent high-intensity efforts

Five of the studies reviewed here were designed to analyze the effects of a single beetroot juice supplement on intermittent high-intensity exercise efforts [38,53,54,57,59]. Aucouturier et al. [54] administered the supplement (~10.9 mmol NO_3^-) to a group of recreation athletes 180 min before performing sets until exhaustion consisting of 15 s of pedalling at 170% VO_{2max}followed by 30-s rest periods. The authors reported that the beetroot supplement gave rise to improvements close to 20% in the number of repetitions performed and the total work completed in the session [54]. Besides the number of sets completed and the work accomplished, these authors measured red blood cell concentrations at the microvascular level. The beetroot juice, apart from improving performance, was found to increase microvascularization. Such improvements are considered a beneficial effect on oxygen exchange in the muscle [95].Accordingly, these oxygen availability improvements produced at the muscular level could have potentiated oxidative phosphorylation during rest periods, and,given their brief duration, could have increased phosphocreatine resynthesis when subjects tookthe supplement rather than the placebo. Thus, supplementation would have delayed the depletion of phosphocreatine reserves and this effect was likely the cause of the improvements observed in the repeated sets of intermittent sprints [96,97].

As did Aucouturier et al. [54], Muggeridge et al. [38] examined the effect of beetroot juice (5 mmol NO_3^-) taken 180 min before an intermittent effort consisting of 5 sets of 10 s in a kayak ergometer with 50-s interset rest periods. In this study, though supplementation seemed to have a greater effect on the power generated in

the last two sets, the improvement noted lacked significance. However, if we compare this study with the study by Aucouturier et al. [54], work periods in the Muggeridge study [38] were shorter (10 vs 15 s) and rest periods were much longer (50 vs 30 s). Ten second maximum intensity intervals have a significantly reduced capacity compared with fifteen second intervals to deplete phosphocreatine reserves. Moreover, the rate of phosphocreatine replacement has a first phase in which up to 50% of these reserves can be replenished in 30 seconds and 100% in 3-5 minutes[67]. Also if we consider that the main effect of beetroot juice supplements is linked to an improved rate of phosphocreatine resynthesis, it is possible that as there is less depletion and a rest period in which there is almost complete recovery of phosphocreatine reserves, supplementation could not have exerted any beneficial effect in the study by Muggeridge et al. [38]. However, despite the short work periods and relatively long recovery periods and the fact that the power developed in the last sets showed an improved trend following supplementation, it is possible that lengthening intervals in a set until exhaustion would have been beneficial and given rise to similar results to those observed by Aucouturier et al. [54].

Rimer et al. [59] assessed the effects of acute supplementation (150 min before exercise) with beetroot juice (11.2 mmol NO_3^-) on performance in a maximal intensity 3-s test on an isoinertial cycle ergometer and a 30-s test on an isokinetic cycle ergometer. Supplementation was effective at improving pedalling cadence, and thus the power generated, in the 3-s test. However, no such effect was observed in the isokinetic test.

The improvements noted by Rimer's group in the 3-s test affected pedalling cadence. Because of the link between such improvements and an increase in muscle shortening velocity [98]and the proposal that NO could increase this velocity [99,100], the authors suggested that beetroot juice could have a beneficial effect on power output [59]. This rationale was also used to explain the lack of changes

produced in the 30-s test in which pedalling cadence was fixed at 120 revolutions per minute. This means that any improved power production in the isokinetic test could only occur if there was an increase in power at a constant shortening velocity[59], since power equals force times velocity.

In a later investigation performed in CrossFit athletes, it was reported that supplementation with NO_3^- salts (8 mmol NO_3) rather than beetroot juice was able to improve performance in a 30-s cycle ergometry test [101]. However, unlike the 30-s test used by Rimer et al. [59], the test was isoinertial. The difference between the two cycle ergometers is that while in the isokinetic test pedalling cadence is prefixed and improvements only in strength are possible, in an isoinertial test the workload is fixedand any power improvements produced manifest as improvements in pedalling cadence. Given that beetroot juice supplementation could improve power development as a consequence of a reduced muscle shortening velocity [59,99,100], the isokinetic cycle ergometer is perhaps not sufficiently sensitive to assess the effects of this supplementation. Considering the beneficial effects on cadence and power output observed in the cycle ergometry 3-s [59]and 30-s[101]tests, it seems that beetroot juice supplementation could have a beneficial effect on this type of effort.

In a fourth study, Clifford et al. [57]assessed the effects of a single intake of beetroot juice on performance in a test of 20 sets of 30 m sprints interspersed with 30-s rest periods. These authors observed no ergogenic effects of the supplementation. However, if we look at the characteristics of the test employed by the researchers, we find that the work periods (close to 3 s) together with the 30 s recovery periods could be sufficient for the subjects to have recovered their phosphocreatine levels in the rest intervals, minimizing the possible ergogenic effects of the supplementation.

A novel indicator used in this study by Clifford et al. [57]was the counter-movement jump (CMJ) test performed before the intermittent velocity test and in the

rest periods. Performance in this test is determined by the contractile properties of muscle and by neuromuscular control of the entire musculoskeletal system[102]. Given that fatigue reflects the incapacity of the neuromuscular system to maintain the level of power required[103], losses in CMJ height at the end of exercise are taken as an indicator of muscular fatigue [104].

In the study by Clifford's group[57], it was observed that the protocol of intermittent sprints gave rise to muscular fatigue. This fatigue can be the outcome of deficiencies in the muscle's contractile mechanism [103,105]. Alternatively, strong eccentric actions of the hamstring muscles during sprints may produce muscle damage [106] and therefore modify the structure of the muscle fibre's sarcomeres. Thus, any loss in CMJ height could indicate muscle damage. While CMJ was monitored after the protocol of 20 sets of 30 mwith30-s rest periods, a greater recovery of CMJ height was observed in the supplementation group. This suggests that beetroot juice could help preserve muscle structure during high-intensity efforts. Another explanation could be related to the vasodilation effect of beetroot juice [50]possibly helping muscle regeneration during early recovery. In future work, biomarkers of muscle damage or inflammation need to be examined.

In the fifth study, Martin et al. investigated the effects of beetroot juice (6.4 mmol NO_3^-) on repetitive sets until exhaustion each consisting of 8 s of work followed by 30 s of rest on a cycle ergometer [53]. No effects were detected on power output in the different sets. Moreover, a lower number of sets was accomplished in the session for the supplementation group versus placebo group. In effect, this was the only study to describe an ergolytic effect of beetroot juice. The authors argued that because of the scarce contribution of oxidative phosphorylation to energy metabolism during high-intensity efforts and that the ergogenic potential of this supplement is related to potentiating oxidative pathways, no beneficial effects are produced on this type of physical action.

The results of the investigation by Martin et al. [53]conflict with those of others who did observe beneficial effects on performance in similar tests [54,56,58,60]. Beetroot juice was taken 120 min before exercise. This regimen is not appropriate, as peak NO_2^-levels are produced 2-3 hour after ingestion and it is recommended that supplementation should be taken at least 150 min-180 min before the high-intensity effort [32, 50]. Effectively, Aucouturier et al. [54] used a test of similar characteristics but the beetroot supplement was taken 180 min before the exercises,as recommended.

Conclusions

To date, few studies have examined the effects of supplementation with beetroot juice on short-duration high-intensity exercise efforts[38,53-60] and observations so far will need confirmation in future studies:

- Supplementation with beetroot juice has been shown to diminish the muscular fatigue associated with high-intensity exercise efforts, though it is not knownif this is achieved by reducing fatigue and muscle damage and/or promoting muscle regeneration postexercise.
- When faced with exercise efforts that could considerably deplete phosphocreatine reserves (sets of resistance training or repetitive sprints of around 15 s interspersed with short rest periods)and given that phosphocreatine resynthesis requires an oxidative metabolism, beetroot juice could help the recovery of phosphocreatine reserves and thus avoid its depletion during repeated efforts. In parallel, supplementation would limit the build-up of metabolites such as ADP and inorganic phosphates, which are known to induce muscular fatigue.
- Beetroot juice has been shown to improve the release and reuptake of calcium at the sarcoplasmic reticulum. This could help the power production associated with improvements in muscle shortening velocity. Non-isokinetic

ergometers (in which movement velocity is not assessed) are sensitive to such improvements in power generation.

Study limitations

The main limitation of our review is the scarcity of studies that have examined the effects of beetroot juice supplementation on intermittent, high- intensity exercise. This limitation is also magnified by the varied design of the few studies available including different supplementation doses and regimens.

Future lines of research

- As it has been proposed that beetroot juice supplementation improves phosphocreatine resynthesis during the brief rest periods included in protocols of intermittent high-intensity exercise, future studies are needed to confirm via a muscle biopsy phosphocreatine levels during repeated high-intensity efforts.
- To examine the possible beneficial effect of beetroot juice on muscle shortening velocity reflected as improvedpedalling cadence, future studies need to assess the ergogenic effect of this supplement in a single, constant-load test on an inertial cycle ergometer.
- To elucidate the mechanism whereby beetroot juice diminishes muscular fatigue and improves recovery from this fatigue, the effects of ingesting NO_3^-on biomarkers of inflammation and muscle damage need to be addressed.
- According to the results of the study in which an ergolytic effect was produced in response to a single dose of beetroot juice administered 120 min before exercise, future investigations should determine the most appropriate timing of supplementation to optimize its ergogenic potential.
- Finally, owing to the possible beneficial impacts of beetroot juice, we will need to assess the interactions of beetroot juice with other supplements of proven

ergogenic effects in this type of exercise effort such as caffeine, creatine, β-alanineand sodium bicarbonate.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data and material

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

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Authors' contributions

R.D. and M.V.G.-G. conceived and designed thereview; E.C., P.G.-F.and F.M.-O. selected the articles included; E.C., M.C.L.-E.and P.V.-H. analyzed the articles included; P.G.-F., F.M.-O. and P.V.-H. translated the manuscript into English; R.D., J.L.M.-M., E.C., S.F.S. and M.V.G.-C. prepared the figures and tables and drafted the manuscript; R.D., J.L.M.-M., E.C., P.G.-F., F.M.-O., M.C.L.-E., P.V.-H., S.F.S.

and M.V.G.-C. edited and revised manuscript; R.D., J.L.M.-M., E.C., P.G.-F., F.M.-O., M.C.L.-E., P.V.-H., S.F.S. and M.V.G.-C. approvedthe final version of the manuscript.

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ARTICULO II

EFFECTS OF A SINGLE DOSE OF BEETROOT JUICE ON CYCLING TIME TRIAL PERFORMANCE AT VENTILATORY THRESHOLDS INTENSITY IN MALE TRIATHLETES

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Abstract

Background: Beetroot juice (BJ) is classified as a high-level supplement for improving sports performance. There is some controversy over the benefits of BJ supplementation for endurance exercise performance, especially when referring to well-trained athletes. This study examines the effects of acute BJ supplementation on cardioventilatory responses, exercise economy/efficiency, slow component of oxygen uptake, time trial performance, blood lactate, energy consumption, and carbohydrate and fat oxidation.

Methods: Twelve well-trained, male triathletes (aged 21-47 years old) were assigned in a randomized, double-blind, crossover design to receive 70 ml of BJ (6.5 mmol NO_3^-) or placebo (PL). Three hours after taking the supplement, participants completed an endurance test on a cycle ergometer at a constant work rate (W) corresponding to first ventilatory threshold (VT1) (30 min) and second ventilatory threshold (VT2) time trial (~15 min).

Results: Maximal oxygen uptake was 54.78 ± 3.13 $mL \cdot min^{-1} \cdot kg^{-1}$, and gross efficiency was>22% at each load intensity and experimental condition. No significant interaction effect (supplement*intensity) was observed on any of the cardioventilatory variables, efficiency/economy, VT2 time trial, energy expenditure, carbohydrate oxidation and fat oxidation ($p > 0.05$).

Conclusion: Our findings do not support an improvement in the variables examined in response to acute BJ supplementation. Probably, higher doses are needed for improving time trial performance in male triathletes during a cycle ergometer test conducted at a load intensity equivalent to the first and second ventilatory threshold

Keywords: cardioventilatory responses; gross mechanical efficiency; cycling efficiency; slow component; energy expenditure.

Background

Beetroot juice (BJ) is classified as a supplement of high scientific evidence for improving sports performance [1]. It is characterized by its high nitrate content (NO_3^-) which, after ingestion, is actively extracted and concentrated in the saliva. Here NO_3^- is reduced to nitrite (NO_2^-) by bacteria in the mouth. In turn, NO_2^- may be further reduced in the stomach and muscle to nitric oxide (NO) [2, 3]. NO is an important signaling molecule with a key role in several physiological processes which may affect exercise performance such as regulating tissue blood flow, muscle contraction, respiration and mitochondrial biogenesis, and muscle glucose uptake [4].

In animal studies, it has been demonstrated that NO_3^- supplementation elevates skeletal muscle O_2 delivery and improves vascular control during exercise predominantly in fast-twitch type II muscles. Furthermore, NO_3^- supplementation improves metabolic control [5]. A human study has suggested that NO_3^- supplementation may enhance physiological and functional responses in type II muscle fibers [6].These potential physiological mechanisms induced by NO_3^- supplementation on type II muscle fibers could justify, at least in part, improvements in performance during intense exercise in healthy adults, and for improving functional capacity in senescent and patient populations [6].

It has been well established that performance at endurance exercise is linked to maximum oxygen uptake (VO_{2max}), lactate threshold, ventilatory threshold (VT), exercise economy/efficiency [7-11]and VO_2 kinetics [12].Acute and chronic supplementation with NO_3^- has been shown to reduce O_2 costin various forms of exercise, with different supplementation protocols and at different exercise intensities. NO_3^- reduced O_2 cost during knee-extensor exercise(6 days, 0.5 l/day NO_3^-, 5.1 mmol/day)[13], decreased during high intensity exercise(6 days, 0.5 l/dayNO_3^-, 5.5 mmol/day)[14] and submaximal exercise (1 day and 15 days,0.5 l/day, 5.2 mmol/day of NO_3^-)[15] in cycle ergometer, and diminished during walking and

moderate- and severe-intensity running(6 days, 0.5 l/day NO_3^-, ~6.2 mmol/day) [16]. In addition, NO_3^-supplementationimprovedmuscle contractile efficiency, increased time to exhaustion by 25 %, reduced the amplitude of the VO_2 slow component by 50%[13],and diminished cycletime trial in trained(6 days, 140 ml/day NO_3^-,~8 mmol/day) [17]and competitive cyclists (1day, 0.5 l NO_3^-,~6.2 mmol) [18]. Because of these findings, it has been proposed that BJ supplementation could have an ergogenic effect in athletes [1]especially when executing long-duration, endurance exercise modalities [19].

There is some controversy over the benefits of BJ supplementation for endurance exercise performance, mainly when referring to highly-trained athletes. In a study performed in elite cyclists, it was found that BJ(6 days, 0.5 l/day NO_3^-, ~0.5 g/day, 820 KJ per drink) failed to improve performance, exercise economy and VO_2 kinetics measured in a 2 h preload test and a 400 kcal time trial[20]. Cermak et al. (2012)[21] also observed that acute BJ intake(500 ml, ~6.2 mmol NO_3^-) did not improve power, time-trial performance or heart rate response in a 1 h cycle time trial in trained cyclists.

International Olympic Committee (IOC) consensus statement [22] determines that improvements in performance after acute BJ supplementation are commonly detected within 2–3 hr following a NO_3^- bolus of 5–9 mmol (310–560 mg) [23]. Longer periods (> 3 days) of NO_3^- supplementation appears to increase sport performance [24, 25], especially, when performance gains appear harder to achieve in highly-trained athletes [12].Higher doses of NO_3^-(> 8 mmol) have shown to improve performance in trained rowers [23].There are some uncertainties for the dose-response relationship exists between biological mechanisms and acute BJ supplementation for improving endurance performance in well-trained athletes. The differences observed between highly-trained competitive athletes and amateur athletes in the effects caused by BJ supplementation could be a consequence of years of training adaptations and genetic factors [26].

The physiological mechanisms underlying the impacts of NO_3^- supplementation on cardiorespiratory endurance performance remain unclear. Studies have shown

that factors such as NO_3^- dose, training level, athlete status, duration of supplementation (acute or chronic), regular dietary NO_3^- intake and exercise test duration and intensity may all affect the impacts of BJ consumption[12]. It remains clear that much further work is needed to elucidate the physiological adaptations and responses induced by BJ in trained or even untrained subjects before and after a training intervention [20].

In the studies performed to date, different test protocols have been used to assess the impacts of NO_3^- supplementation and there is some debate as to which tests are the most suitable for assessing endurance performance. Tests of time to exhaustion measure exercise capacity more than performance per se. These protocols have been criticized for their deficient ecological validity and their limited applicability to some sports modalities[27, 28]. Other studies have based their assessments on covering a given distance in the fastest time possible (time trial) as an intervention to improve sport performance [27, 29].

The arduous nature of laboratory cycling time trials means it is not possible to ask participants to execute a familiarization trial at the criterion distance, which could indicate a lack of knowledge of the performance variability of cyclists in time trials, especially in subjects not experienced in cycling[30]. Given the complexity of designing specific tests to simulate real sports conditions (lab tests vs races), we propose opting for a test conducted at an intensity equivalent to VT, in which aerobic performance (first ventilatory threshold or VT1) and transition towards an anaerobic energy metabolism (second ventilatory threshold or VT2) can be assessed. The use of submaximal VT workloads seems to more accurately predict cycling endurance performance [31]. This has been described as a valid method [32] that shows a direct relationship between VT and 40 km time trial performance. Moreover, the gas exchange threshold and VT are highly correlated with running velocity in triathlon and marathon tests [33-35], and VT2 is a strong predictor of performance in time-trials[36].

The objective of the present study was to assess the effects of acute BJ supplementation on endurance exercise performance and cardioventilatory responses in well-trained triathletes during a cycle ergometer test conducted at a

load intensity equivalent to the first and second ventilatory threshold. Our working hypothesis was that a single dose of BJ supplementation would improve cardiorespiratory endurance performance by diminishing $\dot{V}O_2$ for a given workload by means of more efficient and economic mechanical and energy-producing physiological mechanisms.

Methods

Participants

Participants recruited were 12 well-trained triathletes at the national (N=8) and international (N=4) level (age, 39.3 ± 7.5 years; height, 176.5 ± 7.5 cm; weight, 72.8 ± 6.9 kg; BMI, 23.4 ± 2.2; VO_2max, 54.8 ± 3.1 $mL \cdot min^{-1} \cdot Kg^{-1}$) from different triathlon clubs in Madrid (Table 1). Participation was voluntary though we established the following inclusion criteria: a) national and/or international competition level; b) VO_{2max}>50 $mL \cdot min^{-1} \cdot kg^{-1}$ in cycling; c) no cardiovascular, respiratory, metabolic, neurological or orthopedic disorders that could affect cycle ergometer performance; d) no consumption of drugs or medication; e) no smoking; and f) no nutritional supplements taken in the three months before the study outset (e.g., caffeine, β-alanine, creatinine, sodium bicarbonate, glutamine, etc.). To be classified as well-trained, subjects had to have undergone training for at least 1 h at least 4 times per week and have competed in at least one organized cycle race in the preceding 12 months [30]. Sample size calculation was based on the results of a pilot study with the same study protocol involving 10 sport science students.The calculation of sample size was performed with α= 0.05 (5% chance of type I error) and 1- β = 0.80 (power 80%), andapplying the results provided from previous studies, which used the same [17] or a smaller sample size [18]. A total of 12well-trained triathletes was required for this study to detect differences between both experimental conditions.

The subjects were informed of the study goals and test protocols before giving their signed informed consent for participation. The study protocol received approval from the Ethics Committee of the Universidad Alfonso X El Sabio (Madrid, Spain).

Table 1. Descriptive characteristics of national and international triathletes

	National Level	**International Level**
Participants	n = 8	n = 4
Age (years)	39.4 (8.1)	39.0 (7.6)
Height (cm)	175.0 (7.3)	179.5 (8.1)
Weight (kg)	72.7 (5.1)	73.1(10.8)
BMI	23.8 (2.4)	22.5 (1.6)
VO_2max ($mL.kg^{-1}.min^{-1}$)	53.1 (2.1)	58.3 (1.7)

Data are provided as mean ± standard deviation (SD) Abbreviations used: BMI = body mass index; VO_{2max} = maximum oxygen uptake.

Study design

Participants completed three cycle ergometer test sessions at our Exercise Physiology laboratory. As in previous studies [37, 38], a washout period of at least 72 hours separated the laboratory visits. Sessions were conducted under the same ambient conditions(temperature 20ºC–22.5ºC, relative humidity 42%–52%) and in the same time frame ($\pm$ 0.5 h). Participants refrained from any high-intensity physical effort from 72 h and refrained fromany type of physical exercise from 24 h before starting the first session to the study end. They were allowed to perform low intensity workouts, except 24 hours before the start of the test.

In Session 1, an incremental test until exhaustion was performed on a cycle ergometer. In this test, determination was made of maximum or peak cardioventilatory indices and ventilatory thresholds VT1 and VT2. The power output (in W) eliciting VT1 and VT2 was recorded to determine the workload for the constant test at the intensity of VT1 and VT2 during sessions 2 and 3.

Sessions 2 and 3 were identical and both experimental conditions were compared BJ vs. placebo (PL). In these sessions, supplement assignment was double blind fashion and random. Participants took the supplement given to them, BJ or PL, as soon as they arrived at the lab ensuring that 50% of the triathletes randomly took PL in the first session and BJ in the second or vice versa. This meant that half the participants in each session worked under one of the two experimental conditions. Three hours after taking the supplement, the athletes started with a warm up before conducting an endurance test on a cycle ergometer at a constant workload (W) corresponding to VT1 (30 min) and, without rest, at a constant workload set at VT2 intensity (VT2 time trial) (~15 min). After VT2 time trial, participants answered a few questions to verify whether they were blinded to the supplementation condition. During 3-hour period post BJ ingestion and before beginning the test, the triathletes remained under resting conditions.

Diet and supplementation

As an individual's diet can affect energy metabolism during exercise [39], subjects were given guidelines by a qualified nutrition professionalto ensure that 48 h before each of the test sessions, they followed a similar diet consisting of ~60% carbohydrates (5.5 g carbohydrate per kg), 30% lipidsand 10% proteins.Dietary ingestion was controlled during the 48-hour period before each test session by means of a combination of usual diet and the nutritionist's recommendations.The diet consisted of typical food sources recommended for endurance athletes (e.g., bread, pasta, rice, milk, chicken, tuna, fruit, etc.)considering the energy intake from the PL and BR beverages.

The triathletes recorded their diet for the 48-hr period before the first experimental test and replicated the same diet during the 48 hr before the second trial. Upon arrival at the laboratory on a test day, participants' diaries were evaluated by a nutrition expert to determine compliance with established dietary instructions.

In the case of not complying with the guidelines, the athlete was excluded from the study.

They were also provided with a list of foods with high NO_3^- contents they should avoid at least two days before the study outset (beetroot, celery, ruccula, lettuce, spinach, turnip, endives, leak, parsley, cabbage). Subjects were also instructed to avoid drinks containing caffeine or alcohol during the 24 h before the tests.No caffeine or alcohol intake was allowed during the study for avoiding any interaction with BJ.

Supplementation was given 3 h before the start of each test [19], as it has been established that NO_2^- peaks in blood 2-3 h after the intake of NO_3^- [40]. Each subject took the supplement by drinking the contents of a randomly assigned bottle containing 70 ml (~6.5 mmol, 404 mg of NO_3^-) of BJ concentrate Beet-It-Pro Elite Shot (Beet IT; James White Drinks Ltd, Ipswich, UK) or PL. The PL was a nitrate-depleted sourceand was prepared by dissolving 1 gof powdered BJ (~0.005 mmol, 0.311 mg of NO_3^-, ECO Saludviva, Alicante, Spain) in a litre of mineral water and adding lemon juice to imitate the taste of the commercial supplement.The PL supplementation was prepared by experts in nutrition and dietetics, and pharmacy. Both drinks (BJ and PL) were supplied in an unlabeled, 100-ml, brown glass bottle. During this period before the start of each test, the triathletes did not ingest food and fluids, apart from water, to guarantee hydration status.

Participants were asked to refrain from brushing their teeth or using a mouthwash, chewing gum or sweets that could contain a bactericidal substance such as chlorhexidine or xylitol in the 24 h prior to the test sessions. The reason for this is that the use of oral antiseptics can prevent increased blood NO_2^- levels after the intake of NO_3^- due to their effects on mouth bacteria [41].

All participants were warned of the possible side-effects of BJ, ie, gastrointestinal symptoms and the red appearance of urine and feces.

Cycle ergometer tests

We used an Ergoselect 200 cycle ergometer (Ergoline GmbH, Bitz, Germany) for the incremental and submaximal tests, that was calibrated and adjusted for use with the corresponding pedals and participants' footwear.

To measure the ventilatory variables, we used a gas analysis system (Ergostik, Geratherm Respiratory, Badd Kissingen, Germany) which was calibrated before each test using known O_2 and CO_2 concentrations and low, medium and high flow to calibrate ventilation. Gas exchange data were taken breath-by-breath to obtain the variables VO_{2max}, minute ventilation (VE), ventilatory equivalent for oxygen ($VE \cdot VO_2^{-1}$), ventilatory equivalent for carbon dioxide ($VE \cdot VCO_2^{-1}$), respiratory exchange ratio (RER), end-tidal partial pressure of oxygen and carbon dioxide ($PetO_2$ and $PetCO_2$ respectively). Heart rate was measured by telemetric recording using a transmitter fixed to the chest that sent data to a portable receiver (RS-800CX, Polar Electro OY; Kempele, Finland). Ventilatory and heart rate data were transferred to a PC for subsequent analysis.

Warm-up consisted of 5 min cycling at a light rhythm for the incremental test (Session 1), with subjects selecting the workload and cadence. Next, the triathletes started a ramp test until exhaustion with an initial 50 W load that was gradually increased in 25 W per minute (5 W every 12 seconds). The participants cycled at a self-selected pedal rate of between 70 to 90 rpm. The test was voluntarily terminated by the athletes when cadence dropped to below 70 rpm, or at the point of extenuation.

The $VO_{2max}$was taken as the highest 30-s mean value attained prior to exhaustion in the test [16]. After the test, the criteria used to determine VO_{2max} were[42]: (1) a plateau produced in the VO_2 curve with increases lower than 1.5 mL $\cdot kg^{-1} \cdot min^{-1}$ between 30 s intervals; (2) RER above 1.10; and (3) a heart rate equal to or greater than the theoretical maximum. Maximum heart rate was recorded as the highest value obtained in the incremental test.

In addition to VO_{2max}, two investigators separately identified VT1 and VT2. If there was lack of agreement, the opinion of a third observer was sought. We defined VT1 as the workload at which increases were produced in both $VE \cdot VO_2^{-1}$ and $PetO_2$,

without a concomitant increase in $VE \cdot VCO_2^{-1}$. Similarly, VT2 was determined when increases were produced in $VE \cdot VO_2^{-1}$ and $VE \cdot VCO_2^{-1}$, but this time accompanied by a drop in $PetCO_2$ [43, 44].

Sessions 2 and 3 were preceded by the same warm-up as for the incremental load test. The ensuing test protocol consisted of 30 min of pedaling at a freely selected rate between 70 and 90 rpm at a constant workload equivalent to VT1, plus a VT2 time trial (~15 min), to try to complete the whole test time of ~45 min. Ventilatory data were recorded as means at 30 s time intervals. The workload (in W) was selected for each individual from the VT1 and VT2 values determined in the incremental test.

The slow component of the exercise test was defined as the difference (Δ VO2, in mL . min^{-1}) between VO_2 at the end of exercise and VO_2 at the end of the third minute of exercise at a constant load, both at VT1 and VT2. The values for the end of minute 3 were taken as the mean of VO_2 from 2 min 40 s to 3 min 20 s, while those recorded at the end of exercise were the mean of the VO_2 values obtained for the last 2 minutes [45].

Mean cycling efficiency (CE) at VT1 and VT2 were expressed in $W \cdot L^{-1} \cdot min^{-1}$ while gross mechanical efficiency (GE) was calculated as the ratio of work accomplished per minute (ie, W in $kcal \cdot min^{-1}$) to energy consumed per minute (ie, in $kcal \cdot min^{-1}$), as described elsewhere [46]. Energy expenditure was calculated from VO_2 and the RER using the tables of Lusk**(**VO_2L . min^{-1}· RER expressed in kcal . $L^{-1}O_2$) [47].

The following equations were used to calculate the rates of carbohydrate and fat oxidation [48]:

Carbohydrate oxidation $(g \cdot min^{-1}) = 4.585 \cdot (VCO_2) - 3.226 \cdot (VO_2)$

Fat oxidation $(g \cdot min^{-1}) = 1.695 \cdot (VO_2) - 1.701 \cdot (VCO_2)$.

Blood lactate concentrations were measured in each participant by an experienced investigator using the analyzer Lactate ProTM 2 (Arkray Factory Inc., KDK Corporation, Shiga, Japan). Clean blood samples (5 µl) were obtained from the

index finger of the left hand. Lactate measurements were made: 1) at rest, 2) 30 s before the end of the VT1 stage, and 3) at the end of the test (VT2).

Participants graded their fatigue using the subjective rating of perceived effort [49]at the same time points as the lactate determinations.

Statistical analysis

The Shapiro-Wilk test was used to check the normal distribution of the data, which are reported as mean and standard deviation (SD), mean and confidence intervals (95% CI) or percentage (%). A two-way ANOVA with repeated measures, supplement * intensity (BJ, PL * VT1, VT2, VT1+VT2), was used to compare the effects of the two experimental conditions (BJ vs. PL) on the cardioventilatory, economy/efficiency, and metabolic variables during the constant-load test conducted at the intensity of VT1 and VT2. When appropriate, Greenhouse-Geisser probability levels were used to adjust for sphere city and Bonferroni adjustments were used to control formultiple post-hoc comparisons. A Student t-test for paired data was used to determine differences between BJ and PL. To determine the magnitude of the response to both experimental conditions (supplements) we estimated partial eta-squared (η_p^2). The scale for classification of η_p^2 was 0.01 = small, 0.06 = medium, 0.14 = large. We also calculated the probability of demonstrating the effectiveness of each supplement through statistical power (SP). Significance was set at $p < 0.05$. All statistical tests were performed using the software package SPSS version 19.0 for Macintosh (SPSS Inc., Chicago, IL, USA).

Results

Intake of BJ and PL supplementation was well tolerated by all participants of the study, however, some triathletes showed beeturia (red urine) and red stools.Participants ingested the prescribed dose of BJ and PL as determined by the nutritionist and their dietary interventions were consistent with established dietary guidelines.After the completion of the tests, all subjects were unable to differentiatebetween BJ and PL conditionand, therefore, the triathletes were blinded to the supplementation condition.

Cardioventilatory responses and VO_2 kinetics

The cardioventilatory variables measured in the incremental test until exhaustion (Session 1) are shown in Table 2 and those recorded at VT1 and VT2 in the constant load tests are provided in Table 3.

No significant interaction effect (supplement*intensity) was observed on any of the cardioventilatory variables ($p > 0.05$). The only significant effect found was that of the supplement (BJ, PL) on VCO_2 ($F_{(1, 11)} = 20.155$, $p = 0.001$, $\eta_p^2 = 0.647$, SP = 0.983). No other effects of the supplement were noted ($p > 0.05$). Intensity effects were produced on heart rate ($F_{(2, 22)} = 89.325$, $p < 0.001$, $\eta_p^2 = 0.890$,SP = 1), VO_2 ($F_{(2, 22)} = 51.293$, $p < 0.001$, $\eta_p^2 = 0.823$, SP = 1), $\%VO_{2max}$ ($F_{(2, 20)} = 95.114$, $p < 0.001$, $\eta_p^2 = 0.905$, SP = 1), VCO_2 ($F_{(2, 22)} = 56.529$, $p < 0.001$, $\eta_p^2 = 0.837$, SP = 1), RER ($F_{(2, 22)} = 29.670$, $p < 0.001$, $\eta_p^2 = 0.730$, SP = 1), VE ($F_{(2, 22)} = 127.248$, $p < 0.001$, $\eta_p^2 = 0.920$, SP = 1), VE . VO_2^{-1} ($F_{(2, 22)} = 36.048$, $p < 0.001$, $\eta_p^2 = 0.766$, SP = 1), VE . VCO_2^{-1} ($F_{(2, 22)} = 22.244$, $p < 0.001$, $\eta_p^2 = 0.669$, SP = 1).

Table 2. Cardioventilatory parameters and load obtained in incremental test

Variable	VT1	VT2	VO_{2max}
Power (W)	195.4 (43.3)	282.1 (37.9)	390.3 (52.8)
VO_2 ($L \cdot min^{-1}$)	2.2 (0.4)	3.0 (0.3)	3.9 (0.5)
$VO_2 \cdot Kg^{-1}$ ($mL \cdot min^{-1} \cdot Kg^{-1}$)	26.5 (11.9)	42.1 (4.6)	54.8 (3.1)
VCO_2 ($L \cdot min^{-1}$)	2.1 (0.5)	3.2 (0.4)	4.9 (0.8)
RER	0.9 (0.1)	1.1 (0.0)	1.3 (0.1)
V_E($L \cdot min^{-1}$)	56.3 (10.7)	87.7 (14.2)	167.7 (36.5)
$V_E \cdot VO_2^{-1}$	24.7 (2.4)	28.1 (2.0)	41.2 (4.5)
$V_E \cdot VCO_2^{-1}$	26.8 (2.1)	26.8 (1.9)	32.3 (2.7)

$PetO_2$(mmHg)	90.8 (4.2)	95.3 (3.1)	106.6 (2.8)
$PetCO_2$(mmHg)	32.4 (2.9)	32.4 (2.6)	27.0 (2.4)
HR (beats·sec^{-1})	120.2 (13.5)	146.3 (13.1)	169.3 (12.8)
Intensity (% of VO_{2max})	55.8 (10.2)	75.7 (6.6)	-

Data are provided as mean ± standard deviation (SD) and percentage (%). Abbreviations used: HR = heart rate; $PETCO_2$ = end-tidal partial pressure of carbon dioxide; $PETO_2$ = end-tidal partial pressure of oxygen; RER = respiratory exchange ratio; VCO_2 = carbon dioxide; VE = minute ventilation; $VE{\cdot}VCO_2^{-1}$ = ventilatory equivalent for carbon dioxide; $VE{\cdot}VO_2^{-1}$ = ventilatory equivalent for oxygen; VO_2 = oxygen uptake; VO_{2max} = maximum oxygen uptake; VT1 = first ventilatory threshold; VT2 = second ventilatory threshold.

No significant impacts of the supplements (BJ vs PL) ($p>0.05$) were detected on VO_2 kinetics measured through the slow component. A similar slow component was observed in both experimental conditions throughout the testing protocol in VT1 (BJ: 83 ± 45 mL . min^{-1}; PL: 71 ± 30 mL . min^{-1}) and in VT2 time trial (BJ: 227 ± 144 mL . min^{-1}; PL: 229 ± 129 mL . min^{-1}) (Figure 1).

Table 3. Comparison between beetroot juice (BJ) supplementation and placebo (PL) experimental condition on cardiorespiratory variables

	EC	VT1	VT2	Total (VT1+VT2)	P^1	P^2
HR(beats·sec^{-1})	BJ	130.7 (17.3)	159.6 (11.7)	145.1 (13.8)	0.517	0.485
	PL	129.4 (17.2)	160.0 (14.0)	144.3 (14.0)		
VO_2 (L·min^{-1})	BJ	2.4 (0.4)	3.4 (0.3)	2.9 (0.3)	0.241	0.493
	PL	2.4 (0.5)	3.3 (0.4)	2.9 (0.4)		
VO_2 (%)	BJ	61.8 (11.3)	85.9 (7.6)	73.8 (8.9)	0.253	0.512
	PL	60.5 (12.2)	83.9 (8.3)	72.2 (9.4)		
VCO_2 (L·min^{-1})	BJ	2.5 (0.4)	3.7 (0.4)	3.1 (0.3)	**0.001**	0.579
	PL	2.4 (0.5)	3.5 (0.5)	2.9 (0.4)		
RER	BJ	0.9 (0.0)	1.1 (0.1)	1.0 (0.0)	0.106	0.623
	PL	0.9 (0.1)	1.1 (0.1)	1.0 (0.0)		
V_E (L·min^{-1})	BJ	74.9 (14.6)	127.6 (19.9)	101.2 (15.9)	0.054	0.622
	PL	72.9 (16.8)	125.5 (23.5)	96.8 (16.8)		
$V_E \cdot VO_2^{-1}$	BJ	28.9 (2.1)	36.3 (4.5)	32.6 (2.9)	0.483	0.587
	PL	28.4 (1.9)	36.5 (5.7)	31.6 (2.5)		
$V_E \cdot VCO_2^{-1}$	BJ	28.9 (1.6)	33.4 (3.1)	31.1 (2.1)	0.162	0.573
	PL	29.3 (1.6)	34.2 (4.4)	31.1 (1.9)		

Data are provided as mean ± standard deviation (SD) and percentage (%). Abbreviations used: EC = experimental condition; HR = heart rate; RER = respiratory exchange ratio; VCO_2 = carbon dioxide; VE = minute ventilation; $VE \cdot VCO_2^{-1}$ = ventilatory equivalent for carbon dioxide; $VE \cdot VO_2^{-1}$ = ventilatory equivalent for oxygen; VO_2 = oxygen uptake; VO_{2max} = maximal oxygen uptake; VT1 = first ventilatory threshold; VT2 = second ventilatory threshold. *[1]*Significant differences for supplementation effect. *[2]*Significant differences for supplementation x intensity interaction.

Cycling efficiency, gross mechanical efficiency, lactate, VT2 time trial

The CE, GE and VT2 time trial data are shown in Figure 2. As occurred for the cardioventilatory variables, there were no significant interaction

(supplement*intensity) effects on CE, GE or VT2 time trial ($p > 0.05$). Neither was a significant supplement effect produced on any of the variables ($p > 0.05$). However, as expected, significant intensity effects were produced on CE ($F_{(2, 22)} = 12.824$, $p < 0.001$, $\eta_p^2 = 0.538$, SP = 0.992), GE ($F_{(2, 22)} = 6.495$, $p < 0.001$, $\eta_p^2 = 0.733$, SP = 1), lactate ($F_{(2, 18)} = 24.743$, $p < 0.001$, $\eta_p^2 = 0.733$, SP = 1), and on VT2 time trial ($F_{(2, 20)} = 95.114$, $p < 0.001$, $\eta_p^2 = 0.905$, SP = 1).

Although no significant differences ($p > 0.05$) were found between BJ and PL in VT2 time trial and VT1+VT2 (Fig. 1D), BJ supplementation lead to a shorter VT2 time trial (BJ: 15 min 33 s, PL: 14 min 42 s).

Energy expenditure, carbohydrate oxidation, fat oxidation and RPE

Data related to energy expenditure, carbohydrate oxidation and fat oxidation are shown in Figure 3. No significant interaction or supplement effects on any variable were produced ($p > 0.05$). Significant intensity effects were observed on carbohydrate oxidation ($F_{(2, 22)} = 81.339$, $p < 0.001$, $\eta_p^2 = 0.881$, SP = 1), and energy expenditure ($F_{(2, 20)} = 91.043$, $p < 0.001$, $\eta_p^2 = 0.901$, SP = 1). No significant intensity effect was detected on fat oxidation ($p > 0.05$). No significant interaction or supplement effects were produced on RPE ($p > 0.05$).

Discussion

As far as we know, this is the first study to examine the possible effects of acute BJ supplementation on a constant workload cycloergometry exercise conducted at VT1+VT2 time trial in well-trained endurance triathletes. As VT seems to be the most accurate predictor of endurance performance, especially in cycling[31], this study was designed to test the efficacy of BJ at improving performance during aerobic energy metabolism (VT1) and during the transition from aerobic to anaerobic metabolism (VT2).

Contrary to our working hypothesis, acute BJ supplementation was not observed to improve cardioventilatory responses, mechanical exercise economy/efficiency, slow component, use of energy substrates or performance in these athletes. Even

though national athletes were less trained than international athletes, no positive effect of BJ supplementation was observed in both international and national athletes.

Our VT1 data confirm the results of other studies [50, 51] in which neither were improvements observed in cardioventilatory responses to low-moderate intensity submaximal exercise after supplementation with NO_3^-. Cristensen et al. (2013) [20] reported no GE increase after the intake of 0.5 L of BJ over 6-day periods (0.5 g nitrate per day). Their test protocol involved different work types including work and rest periods in elite cyclists. In another study, Bescos et al. (2011) [50] also detected no GE improvements in well-trained cyclists and triathletes in response to acute sodium nitrate supplementation (10 mg · kg^{-1} dissolved in 250 mL of water), in a test in which there was a single transition at different intensities and with a limited rest period. In contrast, others have shown increases in GE [51] and reductions in pulmonary VO_2 and O_2 cost in submaximal low-moderate intensity exercise in healthy moderately- and well-trained athletes following the intake of BJ [14] (0.5 L for 6 days, 5.5 mmol per day of NO_3^-) and sodium nitrate (0.1 mmol kg^{-1} bodyweight day^{-1}) [51] using different supplementation protocols and cycle ergometry as the assessment test. There is no consensus on the appropriate dose in well-trained athletes at low-moderate exercise intensity.

Previous studies have demonstrated that higher BJ supplementation dose (~8.4 mmol and ~16.8 mmol of NO_3^-)caused a greater reduction in systolic blood pressure and mean arterial pressure at moderate exercise intensity than lower doses (~4.2 mmol of NO_3^-) in healthy adults [37]. In this study, VO_2steady-state of moderate exercise intensity was reduced significantly after ingestion of 16.8 mmol of NO_3^-, tended to be lower after intake of 8.4 mmol NO_3^-, and was unaffected by 4.2 mmol of NO_3^-[37]. Higher doses of BJ supplementation (2 x 70 mL doses per day, ~6.2 mmol of NO_3^- per 70 mL) before and during prolonged moderate-intensity exercise might be necessary to attenuatethe progressive rise in $VO_2$and reduce muscle

glycogen depletion [52], improving mechanical efficiency during a prolonged constant-load test at VT1 intensity. Further, IOC consensus statement concludes that longer periods (> 3 days) of NO_3^- supplementation couldincrease sport performance in highly-trained athletes [22].

It is unclear thatdose-response relationship exists between acute BJ supplementation and the physiological mechanisms for the reduction in the O_2 cost andpulmonary O_2, decrease in VO_2 slow component and increases in GE during low-moderate exercise intensity.Probably, the dose used in our study was not enough to cause an ergogenic effect in well-trained triathletes.

The type and/or mode of supplementation or the test used do not seem to play as important a role as cardiovascular fitness level when assessing VO_2, O_2 cost and GE at low-moderate intensity, as moderately-trained healthy athletes have shown a favorable response to BJ supplementation as opposed to a negative response of well-trained athletes, regardless of the test or supplementation protocol (acute or chronic). Subjects with a lower fitness level may be more susceptible to BJ effects regardless of whether the BJ supplementation is acute or chronic. In effect, the literature suggests some interaction between training state and the ergogenic effects of NO_3^- supplements [53], though the physiological mechanisms induced remain unclear and are likely related to adaptations achieved in response to endurance training [54]. It is known that the most skilled individuals feature better vascular control, characterized by a greater activity and presence of the enzyme endothelial nitric oxide synthase (eNOS), responsible for endogenous NO production [55]. Thus, any increase in eNOS activity could reduce the availability of NO derived from nitrates, consequently diminishing the possible effects of BJ.

This rationale could explain, at least in part, the results obtained here for VT1 as this is a low-moderate intensity of exercise after which a first evident shift is produced in ventilation and in blood lactate concentrations and above which anaerobic energy

metabolism is partly involved [56]. Lactate concentrations in our athletes at VT1 were lower than 2 mmol . L^{-1} indicating a predominantly aerobic state. During this metabolic stage, such intensity of exercise may be maintained over a long period of time without marked changes in blood lactate concentrations [57, 58]. Hence, it is less likely that a trained athlete will experience low muscle oxygenation increasing muscle acidosis and generating nitrate reduction at a given work rate [30]. We suspect there was no tangible effect of BJ, as more trained subjects could show reduced O_2 uptake due to a decrease in the aerobic energy required or in the muscular energy used in moderate exercise efforts.

The reduction in VO_2, attributed to reduced ATP resynthesis through oxidative phosphorylation, was not offset by elevated glycolytic ATP provision[14], as indicated by the similar blood lactate concentrations observed in the groups BJ and PL. However, as argued by Bailey et al. (2009)[14], in less trained subjects, a beneficial effect of NO_3^- is produced reflected by increased muscle oxygenation indices and total hemoglobin levels during moderate exercise. The increased blood volume observed in the vastus lateralis muscle after BJ intake is presumably a consequence of improved muscular vasodilation resulting from the increased production of NO from NO_2^-.

Compared to VT1, physiological and efficiency (intensity effect) changes were observed here in VT2. Studies have shown that BJ enhances high-intensity endurance exercise performance in moderately-trained subjects [14, 59] while its effects are not so clear in well-trained subjects. In a study conducted in elite cyclists [20], the time taken to complete a time trial failed to vary significantly between individuals given BJ or PL. This is similar to the effects on VT2 observed in the present study (400 kcal-time trial 18:20; VT2 time trial 15:33 min:s, respectively), with comparable levels of power reached (290.0 ± 43.0 W vs 282.1 ± 37.9W respectively). Thus, it could be that the intensities set in both tests (preload vs. VT1 and 400 kcal-time trial vs. VT2 time trial) gave rise to an aerobic metabolism and transition to an anaerobic energy pathway.

In statistical terms, BJ showed no endurance performance-enhancing effect in both studies (0.8% and 5.7% in our study). However, significant performance improvements in response to BJ have been observed in well-trained cyclists and triathletes of 1.2%[17] and in rowers (–1.6 ± 1.6 s)[23], along with an increase, though not significant, of ~2% in trained cyclists and athletes[50], and a beneficial response in some elite athletes[60]. Collectively, these findings point to a possible ergogenic effect of BJ on the cardiorespiratory performance of highly-trained endurance sport athletes. It should be considered that to increase the possibility of winning, a high-level endurance sport athlete needs to achieve a gain in total time of at least 0.6% [61]. For example, the variance between twelfth and first place in the 10000m men's running final at the 2012 London Olympics was only 0.66% [62]. Such a slight biologicalimprovement induced by BJ supplementation(not statistically significant),together with intrinsic and extrinsic motivational factors, could be determining factors for success in high-level athletes and this impact may have been detectable in a larger sample size. It would be logical to assume that BJ supplementation could at least partly influence cardiorespiratory performance especially when small improvements in endurance tests can be particularly meaningful. Because these changes in performance are so small, it would be noteworthy to evaluate the differences between physiological and motivational factors produced by BJ supplementation.

Currently, the scientific literature lacks data on the effects of nitrates on high-intensity exercise [53] and the assumption gains importance that BJ supplementation could improve the capacity to cope with fatigue in situations of transition from an aerobic to anaerobic energy metabolism, despite a poor understanding of the physiological etiology involved in well-trained athletes. This is especially true as the slight, yet interesting, increase in VT2 time trial took place in the absence of a beneficial impact of BJ on the cardioventilatory response, exercise economy/efficiency, slow component, use of substrates and blood lactate concentrations.Maybe, an increase in the BJ supplementation dose would have been a factor key to detect improvements in the variables analyzed in our study.

Previous findings have shown that higher BJ supplementation dose (~8.4 mmol and ~16.8 mmol of NO_3^-) improves the time-to-task failure when is compared with a 4.2 mmol dosein young healthy adults [37]. Effectively, in well-trained subjects it would be necessary to intake larger NO_3^- doses (140 mL, ~8.4 mmol, 550 mg of NO_3^-) [23].A normal dose (70 mL) is unlikely to trigger an ergogenic effect. Higher dose (~16.8 mmol of NO_3^-) raises the plasma NO_2^- levels to a greater extent than ~8.4 mmol of NO_3^-, however, no added performance gains are produced [23]. Prolonged periods of BJ supplementation longer than 3 days could increase sports performance [24, 25] and could be used as an alternative supplementation strategy for well-trained athletes.

Furthermore, whole vegetables have been demonstrated to provide important health benefits whereas $NO_3^-$from other sources could lead to adverse effects on health [63]. Because NO_3^- consumed in the form of vegetables have been shown to improve running performance in healthy adults [63], it is tempting to speculate that supplementation strategy based on whole beetroot could be an interesting choice for well-trained competitive athletes while preserving their health.More studies analysing the effects of whole vegetables intake on endurance performance in well-trained athletes are necessary to substantiate such claims.

As a final remark, changes in exercise intensity from VT1 to VT2 involve variations in VO_2, leading to the use of different substrates. Carbohydrates are more efficient as energy substrates than fatty acids. In other words, if more carbohydrates are used as substrate this gives rise to lower oxygen absorption at a given work velocity [51]. Calculations of GE include possible RER changes and, therefore, take into account substrate use. Neither did BJ seem to induce more efficient substrate use as reflected by our data for GE, RER, and consumptionof energy, carbohydrates and fats in both experimental groups. Further, our GE calculations were targeted at

assessing the effects of blood alkalization on gradual losses in muscle efficiency as the best indicator of the so-called slow component phenomenon [64]. With this protocol inducing a change in metabolism from VT1 to VT2, we sought to examine the effects of BJ after promoting a change in VO_2 kinetics (slow component). This change is similar to that observed after an initial bout of high-intensity exercise, giving rise to increased muscle O_2 release, increased oxidative metabolic enzyme activity, carbon substrate availability, and abnormal motor unit recruitment patterns [65, 66].

It is not clear in the scientific literature whether any of these physiological mechanisms could reduce the slow component in response to BJ supplementation in healthy moderately-trained subjects [14, 67]. In a recent study, Tan et al. (2018) [52] demonstrated that BJ supplementation mitigated the progressive rise in VO_2 over time before and during prolonged moderate-intensity exercise although did not enhance subsequent time trial performance. Interestingly, it was observed that this decrease in VO_2 had no impact on time trial performance, which could indicate that supplementation with BJ does not sufficiently reduce muscle glycogen depletion at moderate intensity for decreasing fatigue during cycling time-trial. More research is needed to analyze the BJ supplementation effect on VO_2 kinetics during endurance tests over two hours.

There are some limitations in this study which should be considered. Previous research indicates that the plasma nitrite of the participants should increase to show an ergogenic effect, however, nitrite and nitrate concentrations in plasma were not measured in our study. It seems that doses close to or greater than 8.4 mmol are more adequate to determine the positive effects of BJ supplementation on endurance performance in well-trained triathletes [23].

The small sample size in this study should be taken into account when drawing conclusions from the data. Minimal changes in endurance performance are usually

observed in well-trained triathletes, therefore, large sample sizes should be required to detect significant changes produced by BJ supplementation on cardioventilatory performance.

Although there are several studies that have carried out a similar washout period, it is possible that the washout period established in our study was not sufficient, which could influence the final results.

Conclusions

Our findings do not support an improvement in the variables examined here produced in response to acute BJ supplementation. We have yet to elucidate the possible ergogenic effects of BJ in highly trained athletes. However, the slight (not significant) modifications observed in performance variables such as test duration or maintaining work intensity at a given load in several studies prompts numerous questions as the mechanical and physiological mechanisms analyzed so far do not support these improvements and remain poorly understood.

Our outcomes suggest a need to analyze individual positive responses to this form of supplementation in well-trained athletes.

Abbreviations

ANOVA: Analysis of variance; BMI: body mass index; CE: cycling efficiency; CI: Confidence intervals; GE: gross mechanical efficiency; NO: Nitric oxid; NO_2^-: Nitrite; NO_3^-: Nitrate; η_p^2: partial eta-squared; $PetO_2$: end-tidal partial pressure of oxygen;

$PetCO_2$: end-tidal partial pressure of carbon dioxide; PL: placebo; RER: respiratory exchange rate; rpm: revolutions per minute; RPE: rating of perceived exertion; SP: statistical power; VE: minute ventilation; $VE \cdot VCO_2^{-1}$: ventilatory equivalent for carbon dioxide; $VE \cdot VO_2^{-1}$: ventilatory equivalent for oxygen; VO_2: oxygen uptake; VO_2max: maximum oxygen uptake; VT1: first ventilatory threshold; VT2: second ventilatory threshold; W: Watt.

Ethics approval and consent to participate

The subjects were informed of the study goals and test protocols before giving their signed informed consent for participation. The study protocol received approval from the Ethics Committee of the Universidad Alfonso X El Sabio (Madrid, Spain) according to the principles and policies of the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

Data are presented in the manuscript, further information available upon request.

Competing interests

The authors have no conflict of interest

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Figure captions

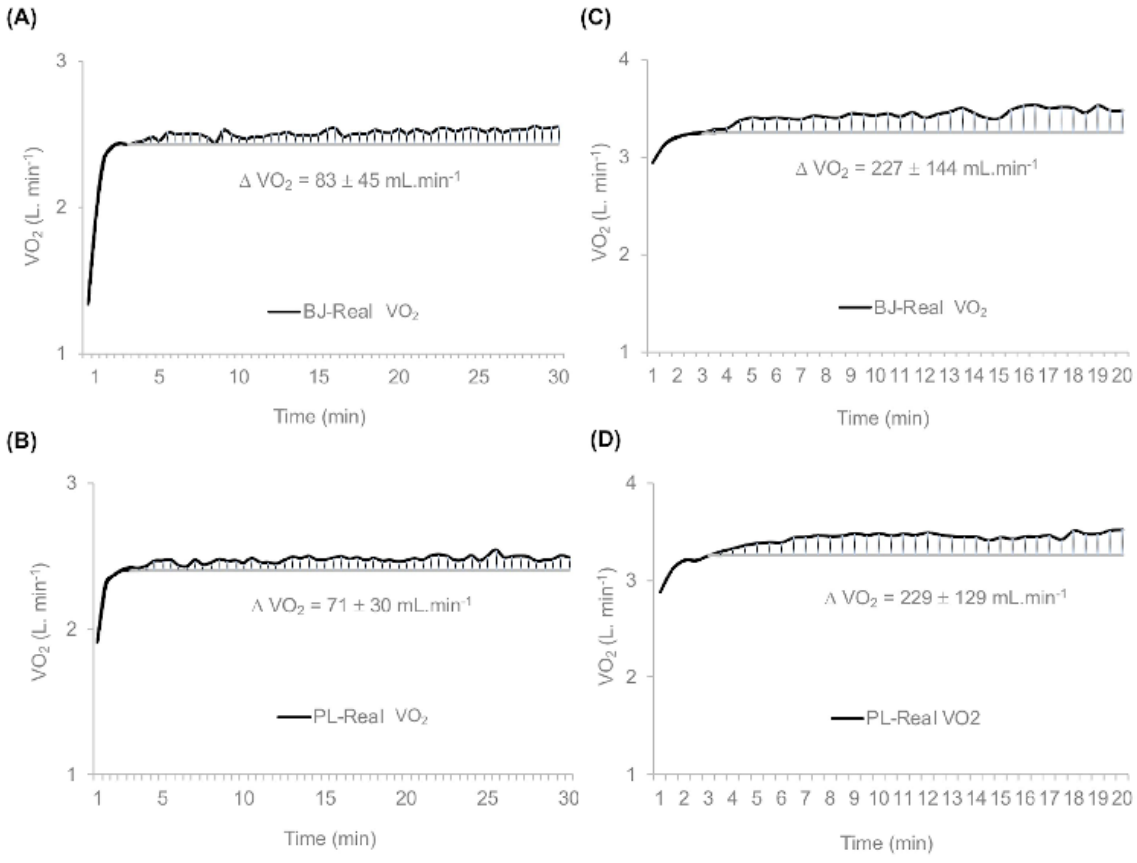

Fig. 1 Slow component (ΔVO_2 mL . min^{-1}) analysis during the constant load test in: (A) Beetroot juice (J) experimental condition at first ventilatory threshold (VT1); (B) Placebo (PL) experimental condition at VT1 (C) Beetroot juice (BJ) experimental condition at second ventilatory threshold (VT2); (D) PL experimental condition at

VT2. Data are provided as mean and standard deviation. There were no significant differences between both experimental conditions (BJ vs. PL) in VT1 and VT2.

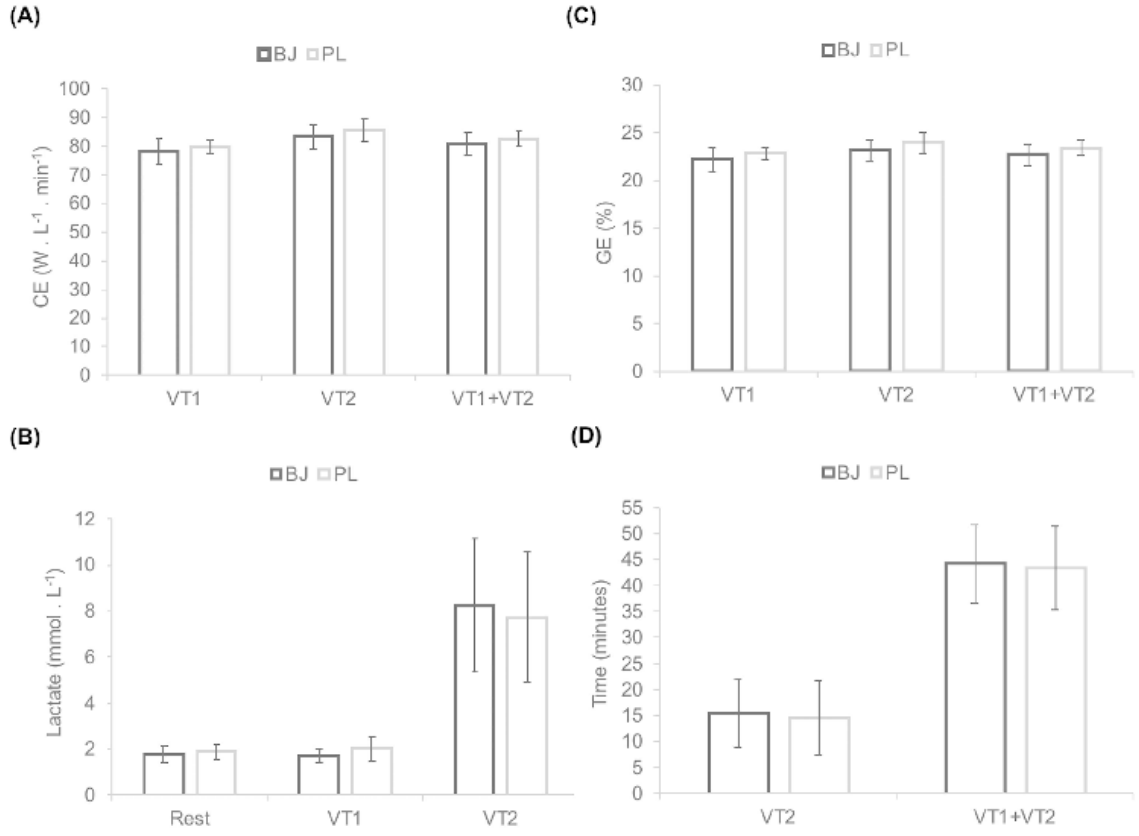

Fig. 2 Differences between beetroot juice (BJ) and placebo (PL) at first ventilatory threshold (VT1), second ventilatory threshold (VT2) and in the total time of the test (VT1+VT2), measured in: (A) Cycling efficiency (CE); (B) Gross efficiency (GE); (C) Lactate; (D) Total time until exhaustion. Data are provided as mean and error bars as 95% confidence intervals. There were no significant differences between both experimental conditions BJ vs. PL.

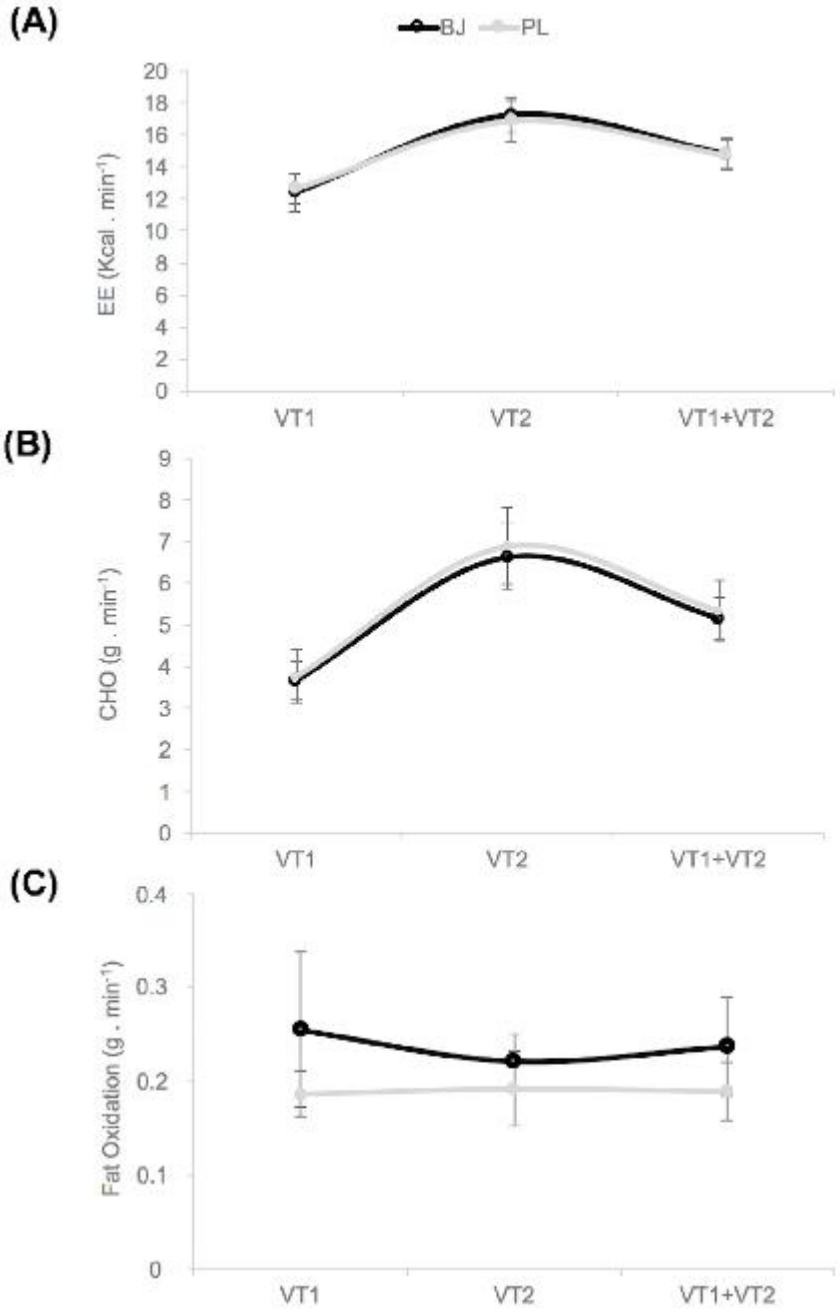

Fig. 3 Differences between beetroot juice (BJ) and placebo (PL) at first ventilatory threshold (VT1), second ventilatory threshold (VT2) and in the time to completion (VT1+VT2), measured as: (A) Energy expenditure (EE); (B) Carbohydrate oxidation (CHO); (C) Fat oxidation. Data are provided as mean and error bars as 95% confidence intervals. There were no significant differences between both experimental conditions BJ vs. PL.

ARTÍCULO III.

EFFECTS OF BEETROOT JUICE SUPPLEMENTATION ON A 30-S HIGH-INTENSITY INERTIAL CYCLE ERGOMETER TEST

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Abstract

Background: Beetroot juice (BJ) is rich in inorganic nitrates and has proved effective at increasing blood nitric oxide (NO) levels. When used as a supplement BJ has shown an ergogenic effect on cardiorespiratory resistance exercise modalities, yet few studies have examined its impact on high intensity efforts.

Objective: To assess the effects of BJ intake on anaerobic performance in a Wingate test.

Methods: Fifteen trained men (age 21.46 ± 1.72 years, height 1.78 ± 0.07 cm and weight 76.90 ± 8.67 kg) undertook a 30-s maximum intensity test on an inertial cycle ergometer after drinking 70 mL of BJ (5.6 mmol NO_3^-) or placebo.

Results: Despite no impacts of BJ on the mean power recorded during the test, improvements were produced in peak power (6%) (p = 0.034), average power 0–

15 s (6.7%) (p = 0.048) and final blood lactate levels (82.6%) (p<0.001), and there was a trend towards a shorter time taken to attain peak power (−8.4%) (p = 0.055). **Conclusions**: Supplementation with BJ has an ergonomic effect on maximum power output and on average power during the first 15 s of a 30-s maximum intensity inertial cycle ergometer test.

Keywords:beet; nitrate; physical activity; sport; supplement

Introduction

Beetroot juice (BJ) is a source of inorganic nitrate (NO_3^-) found in other vegetables or used as preservatives for processed meat products [1]. After the intake of BJ, around 25% of the NO_3^- present is reduced by bacteria in the mouth to nitrite (NO_2^-). As it reaches the stomach, some of this NO_2^- is reduced to nitric oxide (NO) [2], and subsequently absorbed along with the nonreduced nitrite in the gut passing into the bloodstream [3] where blood NO and NO_2^- concentrations rise. Besides this rise in NO levels produced after consuming NO_3^-[4], in situations of low oxygen levels the NO_2^- present in blood may be again reduced to NO [3]. Thus, the final result of taking a BJ supplement is that blood NO levels rise.

NO plays a key role in several physiological, hemodynamic and metabolic events [5]. NO causes blood vessel dilation through mediation by guanylate cyclase [6], increasing blood flow to the muscles and reducing VO_2 at a given work rate [7]. Studies have indeed shown that NO has beneficial effects on muscle contraction [8] and biogenesis [9] and mitochondrial efficiency [10]. Nitric oxide plays a role in efforts that require an oxidative-type of energy metabolism as in endurance exercises performed at a work rate lower than VO_{2max} and of duration longer than 5 min[11].In these high-intensity efforts, many studies—though not all[12–16]—have measured performance or endurance indicators such as economy following the intake of BJ [17–28]. Hence, in endurance exercise modalities, BJ supplementation has been reported to reduce VO_2 at work rates equivalent to theventilatory threshold (VT) [10], first lactate threshold (LT1), second lactate threshold (LT2) [26], 45% VO_{2max}[18], 50% VO_{2max}[25], 60% VO_{2max} in conditions of normal oxygen levels [21] and low levels [24,28], 65% VO_{2max}[18] and 70% VO_{2max}[25,28]. In addition, BJ supplementation has shown an ergogenic effect in cycle ergometry tests until exhaustion executed at

work rates equivalent to 60% VO_{2max}, 70% VO_{2max}, 80% VO_{2max}[13], 90% VO_{2max}[25] and to 70% [20] or 75% [22] between VT and VO_{2max}, as well as improved performance at 4-[29], 10-[23] and 16- km tests in normoxia [17] and hypoxia [24], 50 miles in normoxia [19] and of 30 min in hypoxia [27].

Apart from endurance efforts, other sport modalities exist in which the predominant energy metabolism, rather than involve oxidative energy processes, entails pathways that are independent of oxygen as is the case for explosive or high intensity efforts [30]. Explosive efforts are those lasting under 6 s in which the main energy metabolism pathway is the high-energy phosphagen system and there is some participation also of glycolysis and oxidative phosphorylation [31]. This pathway gradually contributes more to energy production until it accounts for 50% of this at 6 s[31]. High-intensity efforts are those of duration 6 to 60 s that feature a major contribution of glycolytic metabolism and smaller participation of high-energy phosphagens and oxidative phosphorylation [30]. Compared to endurance efforts, these high intensity efforts potentially have an even greater capacity to increase blood NO concentrations in response to BJ supplementation. This is because during the execution of this type of exercise movement, in which the main energy metabolism is independent of oxidation reactions, a drop is produced in the partial pressure of oxygen and pH in muscle and venous and capillary blood [32], and these conditions promote the reduction of NO_2^- to NO [3].

Studies in animals have shown that NO's blood flow improving effect is greater for type II than type I motor units [5,29]. Further, also in animals it has been noted that the power production improvement produced in response to BJ is specific to motor type II units [33]. This is because this type of muscle unit has a greater power production capacity and is designed to obtain energy via non-oxidative pathways. This could be due to the greater capacity of these units to store glycogen and muscular creatine[34], as well as proteins such as carnosine [35], which have a buffering effect at the intracellular level [36]. Thus, BJ intake could have an ergogenic effect during both explosive efforts and high intensity efforts. A 30-s maximum sprint test on a cycle ergometer (Wingate test) can be used to assess performance at high intensity efforts by determining power output and glycolytic capacity [37]. In addition, explosive efforts can be assessed in the first 5 s of the Wingate test, as in this interval

adenosine triphosphate (ATP)resynthesis occurs mainly via the high-energy phosphagen system [38]. Accordingly, in the present study, we examined the effects of BJ supplementation on anaerobic performance in a Wingate test conducted by athletes trained in sports modalities with a high glycolytic energy metabolism component.

Materials and Methods

Participants

Participants were 15 male undergraduates of Physical Activity and Sport Sciences with experience with the Wingate test (they had performed at least one test in the month before the study onset). Descriptive data for the study population are provided in Table 1. Participation in the study was voluntary, though subjects were required to fulfil the following inclusion criteria: (a) more than two years' experience in sports modalities with a high glycolytic energy metabolism component (speed tests in athletic sports and swimming, combat and team sports); (b) not considered an elite athlete; (c) an absence of cardiovascular, lung, metabolic, or neurologic disease or of an orthopaedic disorder that could limit cycle ergometry performance; (d) no medication; (e) no smoking; (f) no nutritional supplements in the six months prior to the study onset.

The subjects recruited were asked to attend a meeting the week before the study outset. In this meeting, three investigators informed them of the study protocol and gave them instructions about diet control and resolved any concerns they had. At the end of the meeting, they all signed an informed consent form.The study protocol was approved by the Ethics Committee of the Universidad Alfonso X El Sabio, Madrid, Spain (code number 1.010.704).

Table 1. Characteristics of the 15 study participants.

Variable	M ± SD
Age (years)	21.46 ± 1.72
Height (cm)	1.78 ± 0.07
Weight (kg)	76.90 ± 8.67
BMI (kg/m^2)	24.21 ± 1.72
Kilogram-force (Kp)	5.77 ± 0.64

BMI = body mass index; M ± SD = mean (± standard deviation).

Study Design

Participants attended two testing sessions at the Exercise Physiology lab within the same time frame (±0.5 h) 72 h apart. From 72 h before the first session until the end of the study, subjects undertook no type of physical exercise. As soon as they arrived at the laboratory, in a random and double-blind fashion, subjects were given a BJ or placebo supplement ensuring that 50% of the subjects randomly took BJ in the first session and placebo in the second or viceversa. This meant that half the subjects in each session worked under one of the two experimental conditions. Three hours after intake of the supplement, subjects started a Wingate cycle ergometer test session including a warm-up.

Nutritional Intervention and Dietary Control

As the blood NO_2^- peak occurs 2–3 h post-ingestion, the supplement was administered 3 h before the endurance test [11]. The use of oral antiseptics can prevent increased blood NO_2^- levels after the intake of NO_3^- because of their bactericidal effect on the bacteria in the mouth. Thus, participants were asked to refrain from brushing their teeth or using a mouthwash, chewing gum or sweets that could contain a bactericidal substance such as chlorhexidine or xylitol in the 24 h prior to the test sessions.

Subjects were also instructed to avoid drinks containing caffeine during these 24 h due to its ergogenic effect [39]. The intake of alcohol was also restricted the day before the study start.

As an individual's diet can affect energy metabolism during exercise, subjects were given guidelines to ensure that 48 h before each of the test sessions, they followed a similar diet consisting of 60% carbohydrates, 30% lipids and 10% proteins and avoiding foods with high NO_3^- contents (beetroot, celery, arugula, lettuce, spinach, turnip, endives, leak, parsley, cabbage). Participants were provided with a list of vegetables they should avoid the day before the study outset.

Each subject randomly took the supplement by drinking the contents of a randomly assigned bottle containing 70 mL of BJ concentrate Beet-It-Pro Elite Shot (Beet IT; James White Drinks Ltd., Ipswich, UK) or placebo. The placebo was prepared by dissolving 1 g of powdered BJ (ECO Saludviva, Alicante, Spain) in a litre of mineral water and adding lemon juice to imitate the taste of the commercial supplement. Although the beetroot juice present in the placebo could have a minimum content of NO_3^-, the small proportion of desiccated beetroot juice in each bottle of placebo (0.015 g), along with the restricted intake of foods rich in NO_3^- 48 h before the start of each session ensured that subjects working under the placebo condition were depleted of NO_3^-.

Both drinks (BJ and placebo) were supplied in an unlabeled, 100-mL, brown glass bottle.

All participants were warned of the possible side-effects of BJ: gastrointestinal problems and the red appearance of urine and faeces.

Wingate Test

The Wingate test was started with the subject stopped. Before the test, the following instructions were given by the investigators: (i) in the first seconds of the test, they should pedal from 0 rpm to the greatest pedalling velocity possible (rpm) in the shortest time possible; and (ii) maintain this high power level during the longest time possible until the test end.

For the test, a Monark cycle ergometer (Ergomedic 828E, Vansbro, Sweden) was used. This ergometer consists of a metal wheel with a band which, through friction, offers resistance to pedalling. This resistance may be regulated as the band is connected to a pendulum that presses on it and this pressure is modified by adjusting a screw under the handle bar. Vertical elevation of the pendulum indicates the kilograms (kg) of friction exerted on the wheel. This friction of the band against the wheel is measured in kilogram force (kgf) defined as the force acting on a 1-kg mass subjected to the acceleration of gravity.

The Monark cycle ergometer has a cog of 52 teeth and a pinion of 14 teeth, causing the conversion of 3.71 revolutions of the wheel for each complete circle of the pedals.The wheel perimeter is 1.62 m and, as for each pedal the wheel spins 3.71 times, the wheel covers 6 m for each complete pedal revolution. The revolutions per minute (rpm) are counted by a magnet system on the pedalling axel and indicated on the speedometer. To calculate the power exerted on the pedals we need to multiply force by the movement velocity:

$$Power = Force\ on\ the\ pendulum\ (kgf) \times Pedalling\ velocity\ (rpm)$$

The force exerted by the band friction is read on the pendulum and expressed in kgf. Velocity is obtained multiplying the rpm of the pedals by the wheel's revolutions. When we multiply the kgf by the metres covered per minute we get kilopondmetres (kpm). To convert kpm into watts (W) one has to divide by 6.12:

$$Kilopondmetres\ to\ watts = 6.12\ kpm = 1\ watt$$

As for the Monark cycle ergometer, each complete pedal circle makes the wheel advance 6 m, each rpm is equivalent to 6 m/min. Thus, using this cycle ergometer, by multiplying the rpm by the kgf indicated by the pendulum, we obtain as a result the power in watts. For data extraction, the display of the Monark cycle ergometer was recorded with a video camera where the rpm during the whole test appeared. Subsequently, the video recording was transferred to the program Kinovea (version 0.8.15, France) which reproduces 30 photo frame/s and the rpm where compiled for each second. Next, we used the equation to determine the watts generated in each second of the test.

Subjects first performed a 5-min warm-up consisting of light cycling with the workload and cadence set by the subject followed by 1 min of rest.After this rest period,subjects executed a specific warm up of 3 min of pedalling at a rate of60 rpm with a workload of 2kgf and a sprint at maximum intensity in the last5 s of each minute. After 3 min of rest, the Wingate test was started.

The test consisted of 30 s of cycling at maximum effort with a load (kgf) corresponding to 7.5% of the subject's body weight [40]. Participants were instructed to pedal as fast as possible to reach the maximum rpm in the shortest time possible and to try to maintain this pedalling speed until the end of the test. Two of the authors motivated the subjects during the test duration. As soon as the test was completed, the subjective rate of perceived exertion (RPE) scale used to rate leg muscle, cardiorespiratory and general perceived exertion.

Just before the warm up and 3 min after the test end, an examiner took a finger prick blood sample (5 μL) from the left index finger for blood lactate determination using a Lactate ProTM 2 LT-1710 blood analyzer (Arkray Factory Inc., KDK Corporation, Shiga, Japan).

Besides blood lactate and muscle, cardiorespiratory and generalRPE, the power (W) variables obtained in the test were analyzed through their transformation of the product ofrpm ×kgf at W.In this way, the variables of W for each second were

examined, obtaining cutoffs for 5-s, 10-s and 30-s intervals during the course of the test. Further variables recorded were: peak power (W_{peak}), time-to-W_{peak}, minimum W (W_{min}), mean power and fatigue index (($W_{peak}-W_{min}$)/W_{peak}× 100).

Statistical Analysis

Initially we confirmed the normal distribution of the data using the Shapiro–Wilk test.

The Student *t*-test for related samples was used to compare the performance variables recorded for the two experimental conditions (placebo and BJ).

All data are provided as the mean (M) and standard deviation (SD). All statistical tests were performed using the software package SPSS version 19.0 (SPSS, Chicago, IL, USA).

Results

Capillary blood lactate levels recorded before (resting lactate) and after the Wingate test (final lactate) and RPE scores after the test are provided in Table 2. The only significant difference detected was an 82.6% higher final lactate level in the group of subjects who took BJ supplements ($p< 0.05$).

Table 2. Metabolic variables and rating of perceived effort recorded in response to the Wingate test according to the experimental conditions (beetroot juice or placebo supplementation).

Variables	Placebo	CV (%)	BJ	CV (%)	%	*T*	*p*
Lactate-resting ($mmol \cdot L^{-1}$)	1.7± 0.45	26.6	2.0 ± 0.53	26.7	15.9	−2.051	0.059
Lactate-final ($mmol \cdot L^{-1}$)*	7.4 ± 2.84	38.0	13.6 ± 4.12	30.2	82.6	−5.337	0.000
RPE-muscular	17.33 ± 1.58	9.2	17.80 ± 1.14	6.4	2.7	−1.388	0.187
RPE-cardiovascular	16.53 ± 2.50	15.1	16.73 ± 1.70	10.2	1.2	−0.315	0.757
RPE-general	17.60 ± 1.88	10.7	17.86 ± 1.12	6.3	1.5	−0.459	0.653

BJ: beetroot juice; RPE = rating of perceived exertion; CV = coefficient of variation; * significant difference for placebo vs. BJ ($p < 0.05$). Data provided as the mean and standard deviation.

The power variables recorded in the Wingate test are shown in Table 3.These data revealed that despite no differences in mean power ($p = 0.796$) between the two supplementation groups, peak power was significantly higher in the BJ vs. placebo group (5.4%; $p = 0.034$) and a trend toward significance was observed ($p = 0.055$) in time-to-peak power (−8.4% for BJ vs. placebo). When comparing power variables recorded at the start and end of the test between supplementation groups, average power 0–5 s was significantly higher in BJ (9.5%; p =0.05), while no differences emerged in average power 25–30 s or in the fatigue index ($p = 0.538$).

When we examined average power in 10-s intervals, average power 0–10 s (placebo = 661.44 ± 113.6 W; CV: 17.2%, BJ = 713.03 ± 116.8 W; CV: 16.4%) was higher in BJ (7.8%; $p = 0.022$), but no significant differences between the conditions were produced in average power 10–20 s (placebo = 677.95 ± 110.2 W; CV: 16.3%, BJ = 708.82 ± 121.3 W; CV: 17.1%) or average power 20–30 s ($p = 0.238$ and p =

0.436 respectively) (placebo = 502.57 ± 99.6 W; CV: 19.8%, BJ = 523.4 ± 106.8 W; CV: 20.4%) (Figure 1). Figure 2 shows that when considering 15-s intervals, a significant difference between BJ and placebo was produced in average power 0–15 s (6.7%; p = 0.048) (placebo = 682.60 ± 108.9 W; CV: 16.0%, BJ = 728.59 ± 118.3 W; CV: 16.2%) but not in average power 15–30 s (p = 0.365) (placebo = 545.36 ± 99.9 W; CV: 18.3%, BJ = 568.24 ± 107.9 W; CV: 19.0%).

Figure 3 visually illustrates how the W values recorded were considerably higher during the first seconds of the Wingate test and reached their greatest values in the upper part of the curve while Figure 4 show the individual and mean group response of the main variables analyzed.

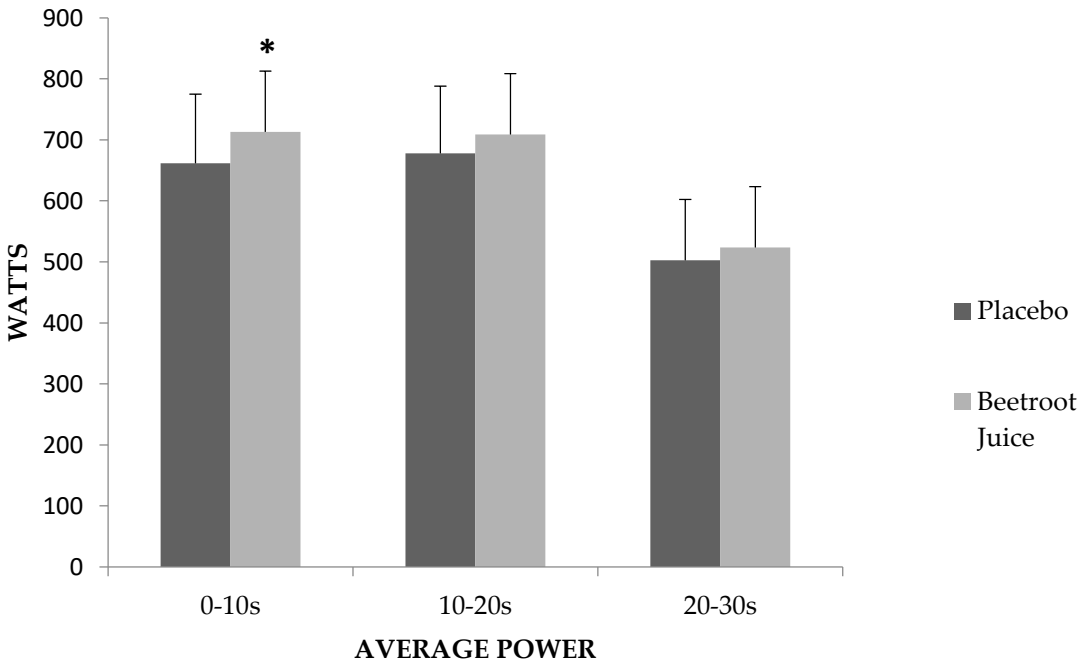


Figure 1. Average power recorded in the intervals 0–10, 10–20 and 20–30 s; * significant difference between beetroot juice and placebo (p< 0.05).

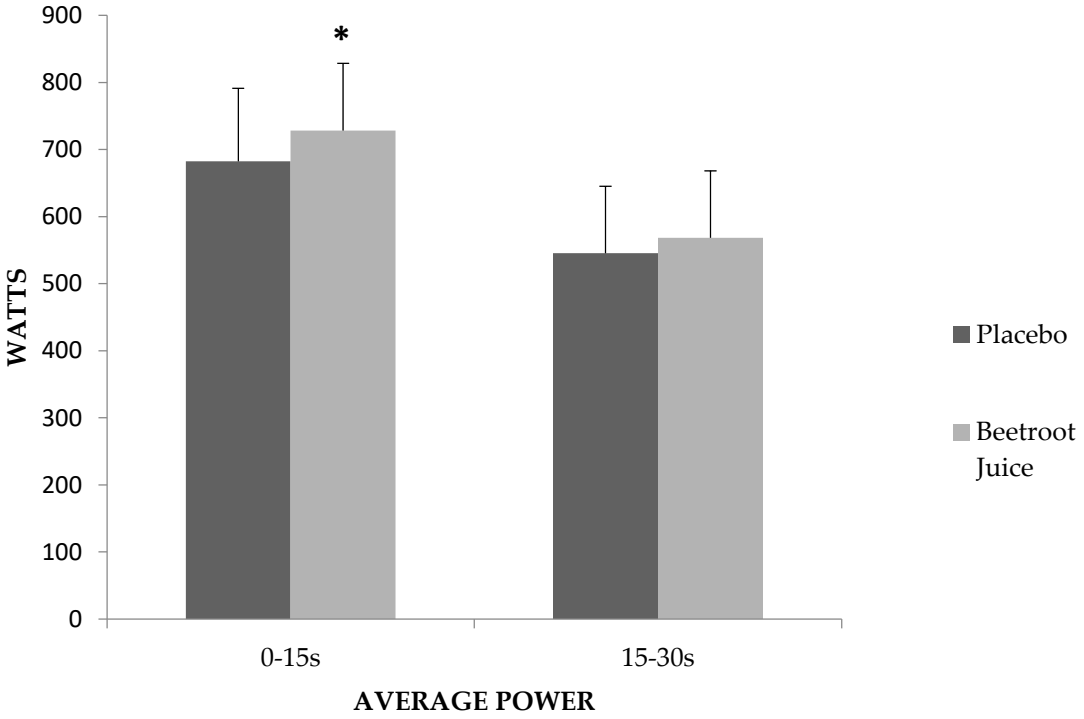


Figure 2. Average power recorded in the intervals 0–15 and 15–30 s; * significant difference between beetroot juice and placebo ($p < 0.05$).

Table 3. Power variables recorded in the Wingate test in participants according to the experimental conditions (beetroot juice or placebo supplementation).

Variables	Placebo	CV (%)	BJ	CV (%)	%	*T*	*p*
Minimun power (W)	433.33 ± 99.39	22.9	442.61 ± 122.79	27.7	2.1	−0.264	0.796
Peak power (W)*	816.83 ± 136.97	16.8	865.69 ± 143.91	16.6	6.0	−2.357	0.034
Mean power (W)	613.98 ± 94.14	15.3	648.41 ± 104.79	16.2	5.6	−1.541	0.146
Time-to-peak power (s)	8.00 ± 1.46	18.3	7.33 ± 1.23	16.8	−8.4	2.092	0.055
Average power 0–5 s (W)*	530.34 ± 106.49	20.1	580.50 ± 109.87	18.9	9.5	−2.141	0.050
Average power 25–30 s (W)	462.46 ± 101.63	22.0	482.28 ± 112.73	23.4	4.3	−0.631	0.538
Fatigue index (%)	46.28 ± 12.01	25.9	48.65 ± 15.54	25.8	5.1	−0.701	0.495

BJ = beetroot juice; s = seconds; W = watts; CV = coefficient of variation; * significant difference for placebo vs. BJ ($p < 0.05$).

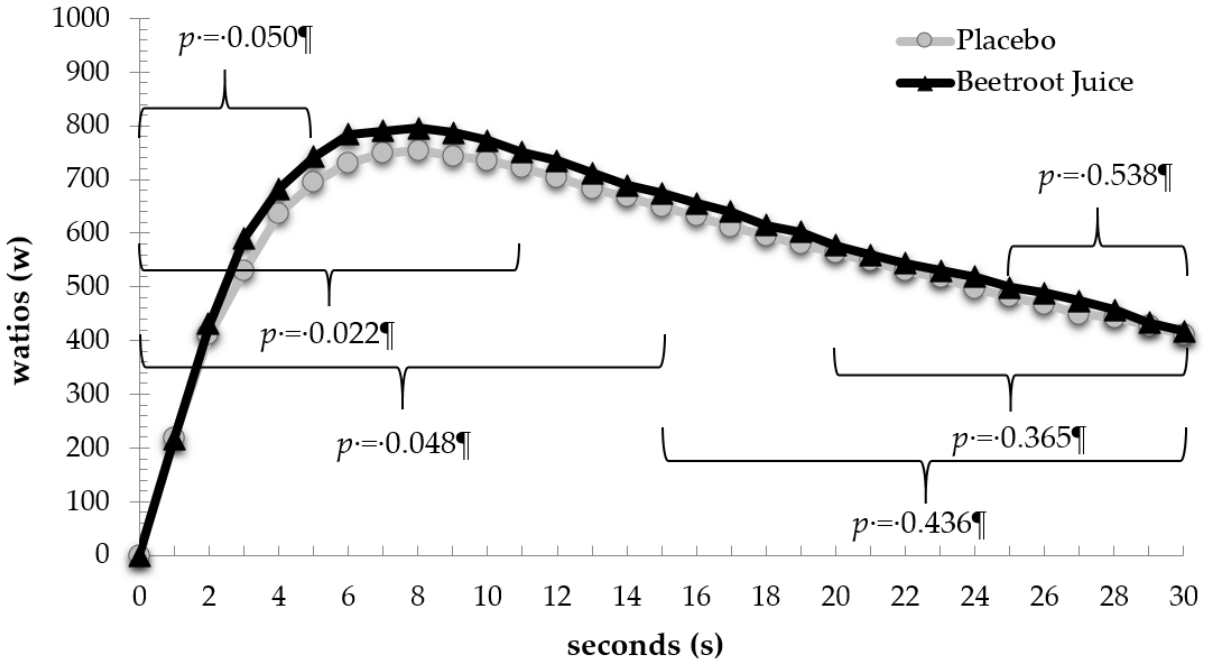
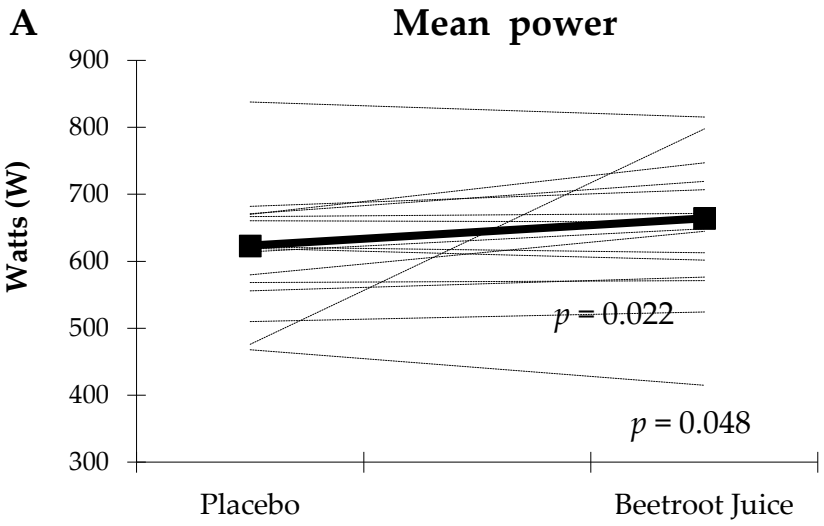
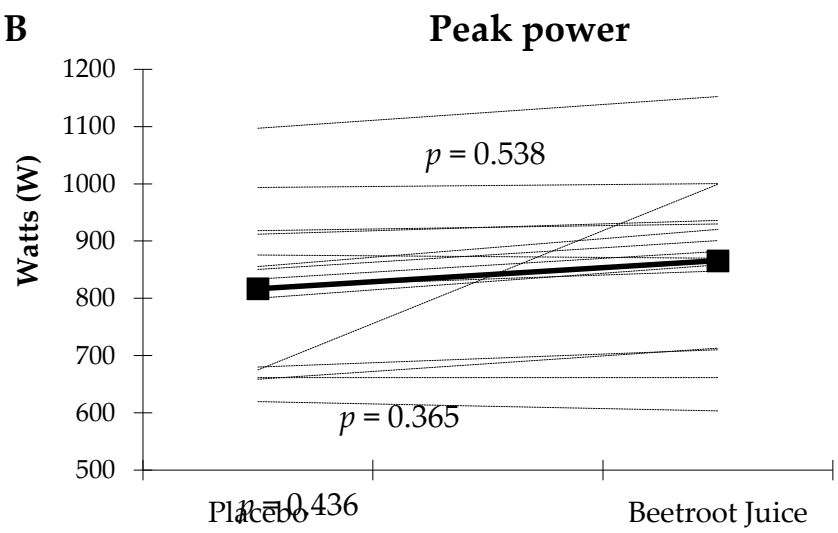


Figure 3. Power curves recorded during the Wingate test in the placebo and beetroot juice supplementation groups. The figure shows that during the first 15 s of the test (0–5 s, 0–10 s and 0–15 s) significant differences in power emerged between the two experimental conditions.


A
Mean power
Watts (W)
900
800
700
600
500
400
300
p = 0.022
p = 0.048
Placebo
Beetroot Juice


B
Peak power
Watts (W)
1200
1100
1000
900
800
700
600
500
p = 0.538
p = 0.365
p = 0.436
Placebo
Beetroot Juice

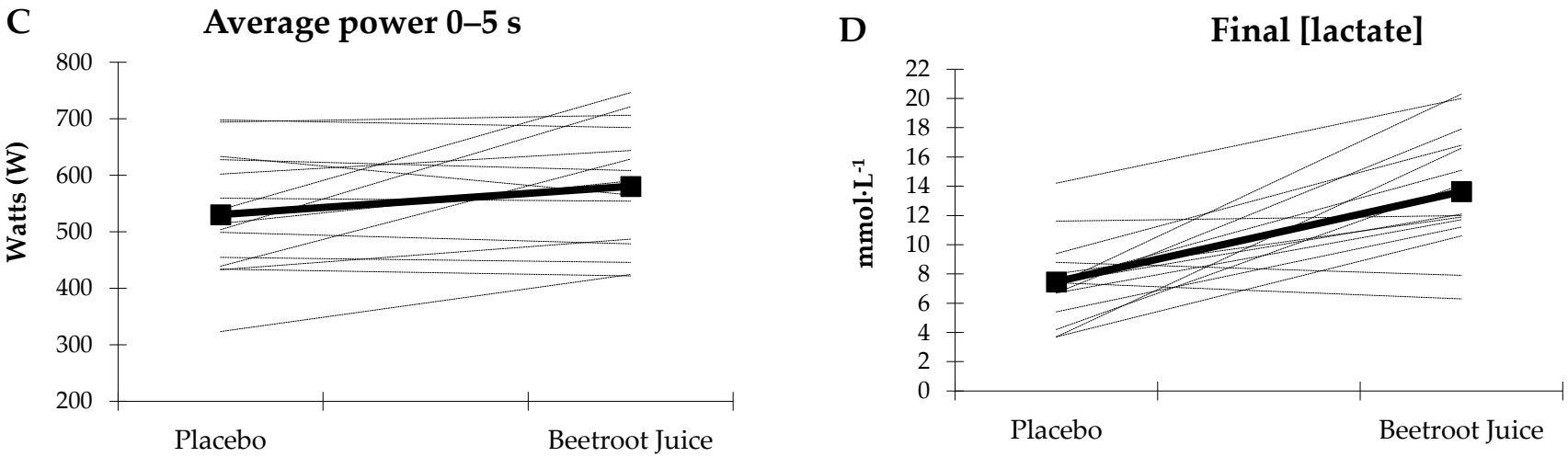


Figure 4. Mean power (**A**); peak power (**B**); average power 0–5 s (**C**) and Final (lactate)(**D**) recorded in all participants (dashed line) and average for the sample (continuous line).

Discussion

The main finding of our study was that BJ supplementation was able to significantly improve the power developed during the first 15 s of a Wingate test, with impacts on W_{peak} and a trend towards a shorter time-to-peak power (p = 0.055). This improved peak power production produced after the intake of BJ (6%) coincides with the results reported in studies examining its impacts on knee-extension exercises (6%) [41]and on inertial cycle ergometry (6%) [42], though the dose used in our study was 5.6 mmol NO_3^- vs. 11.2 mmol NO_3^- used in other investigations [41,42]. However, in this last study, BJ supplementation led to no such improvements in average and peak power during a Wingate test executed on an isokinetic cycle ergometer rather than an inertial one as in the test employed here [40].

Despite reports of significantly raised NO_2^-levels in response to BJ doses of 8.4 mmol NO_3^- and 16.8 mmol NO_3^- compared with a dose of 4.2 mmol NO_3^-[43], our finding of improved peak power attributable to the intake of 5.6 mmol NO_3^- BJ before the Wingate test supports the results described by Rimer et al. [42] in response to a dose of 11.2 mmol NO_3^-. To explain the lack of an effect of BJ on an isokinetic Wingate test, we need to consider the characteristics of the different ergometers used in the study by Rimer's group. As power reflects the relationship between force and velocity, for an inertial cycle ergometer in which the load remains constant (fixed at a load relative to a percentage of body weight) [44], any changes produced in power production are attributable only to modifications in pedalling cadence [45]. In contrast, when performing the test on an isokinetic cycle ergometer, the pedalling cadence is fixed and power is interpreted as the force exerted at a given velocity. Because pedalling cadence is known to correlate highly with knee and hip angular velocities, this cadence is used to indicate the shortening velocity of the muscles involved in both these joints [45]. Hence, improvements in the power produced when pedalling on inertial cycle ergometers are sensitive to changes in power output associated with velocity, while improvements when using an isokinetic cycle ergometer are related to variations in force. As one of the functions of NO is to reduce muscle shortening

velocity [46], the beneficial effects of BJ supplementation may perhaps not be observed when using an isokinetic ergometer.

Among the factors that affect the production of power, we should highlight the influence of the type of muscle motor unit recruited, as type II muscle fibres show a greater contraction velocity and force [47]. Accordingly, it has been observed that the improved peak power produced following BJ intake is specific to type II motor units [48]. Studies in animals have also shown that the effects of BJ supplementation on blood flow [5] and force production [33]are only observed in type II motor units. In effect, studies in animal models have shown that NO increases the effects of acetylcholine exclusively in type II muscle fibres[49]. An improved action of acetylcholine may enhance motor neuron depolarization [49]. Besides, BJ increases the expression of calsequestrin[29], increasing calcium release from the sarcoplasmic reticulum to the muscle fibre sarcoplasm [50]. At this site, calcium binds to tropomyosin and troponin promoting actin and myosin crossover [51]. Increased action potential succession and the presence of calcium could promote trains of action potentials thus increasing peak power output [52]. In effect, this has been observed by monitoring electromyographic activity during maximum intensity efforts [53].

In addition to the effects of BJ supplementation on force production in type II motor units, there have been reports that BJ reduces ATP demands during the exercise effort [4,54], manifesting as the reduced degradation of phosphocreatine (PCr) both in low and high intensity exercise [54]. A diminished PCr cost during the maximum intensity effort would delay the depletion of PCr reserves [6]. Given the essential role of PCr in high intensity efforts [20], its delayed depletion during the Wingate test should help maintain greater power peaks during the first part of the test, thus explaining the significant improvement noted in average power 0–15 s (6.7%).

The effects of BJ reported here are consistent with those of a study in which the effects ofsupplementation with nitrate salts were examined in a Wingate test, also performed on an inertial cycle ergometer in a population of similar characteristics (CrossFit athletes). Thus, it was observed in CrossFit athletes that

supplementation with 8 mmol of potassium nitrate led to a 6.6% improvement in peak power [55], comparable to the present finding of 6%. Also, while no significant improvement was observed in the average power developed during the test, a trend towards significance was noted (p = 0.08) [55]. However, as these authors did not compare power production across test intervals, it is not known whether a significant improvement was produced during the first 15 s of the test as noted in our study.

The effects of BJ [42]or nitrate salts [46] on maximum power produced during cycle ergometry mediated by increased NO concentrations or reduced PCr degradation rate [5,54] could also explain the findings of several studies: a greater number of repetitions (26.1 vs. 21.8) of 15-s bouts of cycle ergometry executed at 170% of maximum aerobic power (MAP) with 30-s rest periods [41]; improved power developed during 24 sets of 6-s work periods and 24-s of rest (~7%)[56]; or improved cycle ergometry work accomplished in 5 sets of6 s followed by 14 s of rest (~3.5%) in the middle and end of a protocol consisting of 2 × 40 min that simulated the demands of a team sport's match [23].

The increase observed here in blood lactate concentrations (82.6%) in response to BJ supplementation is similar (106.3%) to that detected in rats given an injection of NO_2^-[57]. Blood lactate concentrations are considered to indicate the glycolytic contribution to energy metabolism [54], though the transfer of lactate to the bloodstream depends on the extent of capillarization and muscle perfusion. In a 30-s maximum load test such as the Wingate, in which type II motorneurons are recruited and there is a highly glycolytic metabolism, blood lactate concentrations are much lower than those of muscle lactate and several minutes are needed for blood and muscle concentrations to reach a balance [58]. It is possible that increased blood flow to type II motor units following BJ supplementation [5], could have led to increased blood lactate concentrations [59]. This possible effect of BJ [5] could be responsible for the increase in blood lactate levels observed after the high-intensity effort in our study and in the study by Glean et al. [57].

Another possible explanation for the increase produced in blood lactate concentrations could be elevated glycolytic activity [57]. Accordingly, the increased power produced in our study would be the consequence of a glycolytic type metabolism during the exercise effort. This mechanism could explain the ergogenic effect of this supplement detected in our study and in others [54]. Wylie et al. [56] observed increased blood lactate levels along with improved performance at a cycle ergometer protocol consisting of 24 sets of 6 s and 24-s rest periods, while Mosher et al. [58] noted an increased number of repetitions accomplished until exhaustion when lifting a load equivalent to 60% of one maximum repetition (1 RM) during bench press exercise. As the impacts on power output of BJ supplementation are attributed to the improved performance of type II motor units characterized by a greater dependence on glycolytic energy metabolism, this leads to a greater increase in blood lactate following the exercise effort [59]. In any case, neither of these two explanations are exclusive such that it could be that the increase in blood lactate produced at the end of the test were the consequence of a greater amount of work executed by type II motor units as well as their greater blood supply.

Limitations

Given that the effects of BJ supplementation are mediated by its capacity to raise levels of NO_2^- which later may be reduced to NO, a limitation of our study was that we were unable to measure blood NO_2^- levels. Moreover, since prior studies have shown a greater effect of BJ doses of 8.4 and 16.8 mmol NO_2^- vs. 4.2 mmol NO_2^- to increase blood NO_2^- levels and improve enduranceperformance [43], we could have compared the impacts of the dose of 5.6 mmol NO_2^- employed with that of a higher dose (11.2 mmol). The increased blood lactate concentrations observed here and in other studies [57] could either be due to a potentiating effect of BJ on blood flow which would accelerate the passage of lactate to the blood or to an increase in glycolytic activity. Hence, by monitoring blood lactate kinetics after the Wingate test, we could have examined whether these higher lactate concentrations persisted during the recovery period (due to increased glycolytic activity) or if the lactate concentration differences would have evened out (indicating increased flow of muscle lactate to the bloodstream as the consequence of an effect on the blood supply to the muscles). A further limitation

is that we did not undertake a test-retest. However, to avoid the possible interaction of the factor time, we randomly assigned the experimental conditions ensuring that 50% of the subjects worked under one or other condition in each test session. In addition, as an inclusion criterion, participants had experience with the Wingate test.

Conclusions

Beetroot juice (containing 5.6 mmol NO_3^-) taken as a supplement had an ergogenic effect on maximum power production and a trend was observed for this to occur within the first 15 s of an inertial cycle ergometer Wingate test. The supplement also led to increased blood lactate concentrations post-exercise. We attribute these effects of BJ to specific improvements in power output and blood supply to type II motor units.

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ARTÍCULO IV.

EFFECTS OF BEETROOT JUICE SUPPLEMENTATION ON PERFOMANCE AND FATIGUE IN 30-S ALL-OUT SPRINT EXERCISE: A RANDOMIZED, DOUBLE-BLIND CROSS-OVER STUDY.

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Abstract: As a nitric oxide precursor, beetroot juice (BJ) is known to enhance high-intensity exercise performance (80–100% VO_{2max}) yet its impacts on higher intensity sprint exercise (>100% VO_{2max}) remain to be established. This study sought to examine the effects of BJ supplementation on performance and subsequent fatigue during an all-out sprint exercise.Using a randomized cross-over, double-blind, placebo-controlled design, 15 healthy resistance-trained men (22.4 ± 1.6 years) ingested 70 mL of either BJ or placebo. Three hours later, participants undertook a 30-s all-out Wingate test.Before and after the

sprint exercise and at 30 s and 180 s post-exercise, three countermovement jumps (CMJ)were performed and blood lactate samples were obtained.Compared to placebo, BJ consumption improved peak (placebo vs. BJ, 848 ± 134 vs. 881 ± 135 W; *p* = 0.049) and mean(641 ± 91 vs. 666 ± 100 W; *p* = 0.023) power output and also reduced the time taken to reach W_{peak} in the Wingate test (8.9 ± 1.4 vs. 7.3 ± 0.9 s; *p* = 0.003). No differenceswere detected in the fatigue index. In addition, while over time CMJ height and power diminished (ANOVA *p* <0.001) and blood lactate levels increased (ANOVA *p* <0.001), no supplementation effect was observed.Our findings indicate that while BJ supplementation improved performance at the 30-s cycling sprint, thisimprovement was not accompanied by differences infatigue during or after this type of exercise.

Keywords:nitric oxide; nitrates; muscle power; muscle fatigue

Introduction

Dietary nitrate supplementation has been described as a potential ergogenic aid for high-intensity exercise efforts (80–100% VO_{2max}) as it reduces the oxygen cost of ATP synthesis and ATP cost of muscle contraction thus improving muscle contraction/relaxation, force and power production [1–3]. However, the impacts of nitrate supplementation on all-out sprint exercise performance (>100% VO_{2max}), and particularly its effects on the fatigue induced by this mode of exercise [4–6] have been scarcely addressed.

Ingested nitrate (NO_3^-) is a well-known precursor of nitric oxide (NO) in humans [7]. Around 25% of circulating NO_3^- is taken up by salivary gland acinar cells in a process facilitated by sialin [8,9]. Oral microorganisms, particularly those on the posterior aspect of the tongue, initiate the reduction of NO_3^- into nitrite (NO_2^-), which subsequently in the stomach and gut, can be converted into NO and be absorbed under hypoxic conditions [8–10]. The majority of the remaining NO_3^- and NO_2^- molecules that reach the intestine are absorbed by this organ increasing NO levels in blood [9].NO offers several exercise adaptation benefits [11] through its effects of inducingvasodilatation, reducing blood viscosity, and

promoting muscular oxygenperfusion and gas exchange [12]. In skeletal muscle, NO reduces oxidative stressor production and promotes mitochondrial biogenesis and efficiency [13,14]. Moreover, NO it is also able toincrease force and power production during muscle contraction, decreasing the cost of ATP needed as well as the oxygen required to synthesize ATP [1–3].

Beetroot juice (BJ) is a NO_3^--rich supplement commonly used because of its high betacyan in and polyphenol contents that promote NO synthesis to a greater extent than other NO_3^- salts [15,16]. The ergogenic effect of NO_3^- supplementation was initially observed in terms of metabolic adaptations to endurance training [17]. However, despite the known impact of BJ on aerobic performance, recent data indicate a potential effect of NO_3^--rich supplements on anaerobic exercise [4].

Interestingly, the observed benefits of BJ only seem to affect type II muscle fibers [11].In these fibers, NO stimulates calcium release into the sarcoplasm via calsequestrin upregulation [18] and reducesthe phosphocreatine degradation rate, decreasing ATP cost across several ranges of exercise intensity [19]. During sprint exercise (>100% VO_{2max}), type II muscle fibers are mainly recruited to satisfy the high muscle contraction demands. In these glycolytic fibers, exercise leads to a reduced pH in comparison to oxidative fibers. Intra-cell acidity also promotes the reduction of NO_2^- to NO [8].In turn, the increase in NO availability may diminish the ATP and phosphocreatine required by each muscle contraction with the consequence of an ergogenic effect of NO_3^-supplementation in sprint exercise achieved by improving power production and attenuating the fatigue induced by this exercise mode [20,21].

However, despite acute BJ administration emerging as an effective strategy to improve different modes of exercise performed to exhaustion [22], the influence of this supplement has been scarcely explored in sprint exercise [1–3,20,23,24]. Two studies have shown that BJ supplementation increases peak power output in a 3–4 s[23] or 30 s cycle ergometer exercise [20,23,24]. However, the benefits of BJ on the muscle power produced in a vertical jump have not been investigated. The countermovement jump (CMJ) is a useful test to explore the

muscle contractile properties and neuromuscular performance of the lower-limbs [25].This test has been extensively used in high-intensity sports in which the stretch-shortening cycle plays a pivotal role [26].Further, given thatfatigue can be defined as a reduction in strength or power regardless of the ability to sustain a required task [27], conducting the CMJ before and after an extenuating task is an effective method of monitoring muscle fatigue [28]. In this context, the present study was designed to examine the effects of BJ, as a NO_3^--rich supplement, on performance at a single 30-s all-out sprint exercise and the fatigue caused by the exercise bout. Our working hypothesis was that BJ intake would increase the peak power generated by muscle contraction and reduce the time needed to achieve this peak power output with the consequence of diminished neuromuscular fatigue after the sprint.

Materials and Methods

Participants

Fifteen young men (age 22.4 ± 1.6 years, height 178 ± 6 cm, weight76.9 ± 10.3 kg) were recruited. All subjects had at least 18 months of experiencewith resistance exercise, training 3 sessions per week (e.g., bench press and leg press 1RM were 1.0 and 1.5-fold higher than their body mass weight, respectively) and were familiar with the 30-s all-out Wingate and CMJ tests. Subjects were instructed to refrain from taking sports supplements, medical supplements or any ergogenic aids during the 3 months before the tests and were excluded if they failed to comply. Further exclusion criteria were smoking or cardiovascular, pulmonary, metabolic or neurologic disease.

Candidate participants were first informed of the experimental protocol before giving their written consent. The study was approved by the Ethics Committee of Alfonso X University in (code 1.010.704) accordance with the latest version (7th) of the Declaration of Helsinki.

Experimental Design

The study design was randomized cross-over, placebo-controlled and double-blind. Participants reported to the laboratory on two separate days under the same experimental conditions (72h between sessions, 0.5h difference in test initiation). Participants were instructed to avoid any form of exercise in the 72h leading up to each test.

In session 1, participants were subjected to a preliminary assessment of body composition and underwent a familiarization session of the experimental protocol. Then, on two separate occasions (sessions 2 and 3)as they arrived at the laboratory, participants were provided with a supplement containing either placebo (placebo) or BJ. The trial was double-blinded such that one researcher (P.V.-H.) allocated all the participants' drinks in a counter-balanced fashion (in each trial 50% of participants ingested placebo and 50% ingested BJ beverages) with random assignment to each supplement (using Excel, Microsoft, Washington, DC, USA) and this researcher did not take part in the subsequent experimental procedures or statistical analysis of data.Three hours after taking the supplement, all participants performed a 30 s all-out Wingate test on a Monark ergometer (Ergomedic 828E, Vansbro, Sweden), as previously described [19]. Strong verbal encouragement was provided in all the sprint tests. In addition, data were collected in three CMJ jumps and blood samples for lactate determination were obtained in duplicate before (Pre) and after the sprint exercise at 30 s (Post) and 180 s post-exercise (Post-3). The study procedure is illustrated in Figure 1.

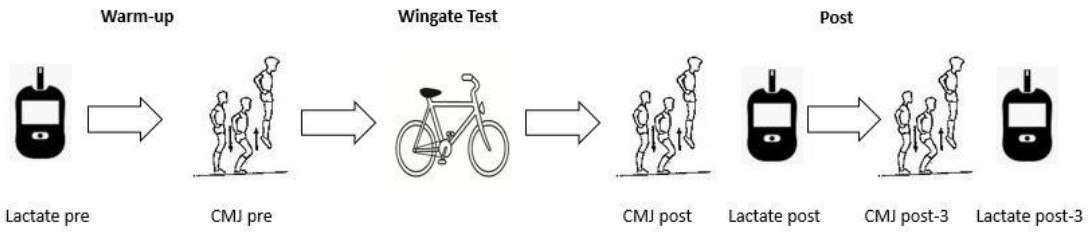

Figure 1. Experimental procedure.

Placebo vs. BJ Ingestion

After an overnight fast, participants reported to the laboratory 3h before the first CMJ jump test. Upon arrival, they were provided with either 70 mL of BJ (containing 6.4 mmol of NO_3^-) or the same drink lacking NO_3^-(placebo, 0.04 mmol of NO_3^-) (Beet-It-Pro Elite Shot, James White Drinks Ltd., Ipswich, UK) as described elsewhere [20].

All participants were instructed to follow a diet sheet the day before each trial that consisted of 60% carbohydrates, 30% fat and 10% proteins. Dietary NO_3^- was limited by providing subjects a list of NO_3^--rich foods (e.g., beetroot, celery or spinach) they should avoid in the 48h before, each trial. Also, in the 24h leading up to each test, subjects were encouraged to avoid brushing their teeth or use an oral antiseptic rinse, or ingest gum, sweets or stimulants (e.g., caffeine) that could alter the oral microbiota and interfere with NO_3^- reduction.

Sprint Performance Variables

Power output (W) was monitored second-by-second in all sprints. Mean power output (W_{mean}) was calculated as the average power generated during the 30-s test. Peak power output (W_{peak}) was taken as the highest W value recorded.The time (s) taken to reach W_{peak} was also recorded. Minimum power output (W_{min}) was considered as the lowest W value recorded during the 10 last seconds of the test. Finally, the fatigue index (FI) was calculated using the equation: $FI = (W_{peak} - W_{min})/W_{peak}$.In addition, mean power output in each Wingate test was calculated for the entire test (30s) and at 10 s ($W_{mean0–10s}$, $W_{mean10–20s}$ and $W_{mean20–30s}$) and 15 s intervals ($W_{mean0–15}$ and $W_{mean15–30s}$) as describedelsewhere [19].

Neuromuscular Fatigue

Neuromuscular fatigue in the legs was measured as the loss of height and power in a CMJ test performed on a force platform (Quattro Jump model 9290AD; Kistler Instruments, Winterthur, Switzerland)[28–30].Participants were highly familiarized with this vertical jump test. Two CMJ were performed before (Pre) and after the Wingate test at 30 s (Post-1) and 180 s post-exercise (Post-3). At

each time-point, mean values of height (cm), mean power (CMJ_{Wmean}) and peak power (CMJ_{Wpeak}) were recorded.

Blood Lactate

Before the first CMJ and immediately after the subsequent vertical jumps, capillary blood samples (5 µL) were obtained from the index finger of the right-hand for lactate determination using a Lactate ProTM 2 LT-1710 Instrument (Arkray Fatory Inc., KDK Corporation, Shiga, Japan).

Statistical Analysis

The Shapiro-Wilk test was first performed to assess the distribution of the data. Then paired *t*-testsfor normally-distributed data and the Wilcoxon test for non-normally distributed variables (Time-to-W_{peak}, $W_{0–15s}$, $W_{15–30s}$, $W_{10–20s}$ and $W_{20–30s}$)were used to compareall sprint variables between the experimental conditions (placebo vs.BJ). A two-way ANOVA for repeated measures was also used to compare placebo vs.BJfor two between-subject conditions: supplementation (placebo vs.BJ) and time (pre-exercise, 30 s post-exercise and 180 s post-exercise).Before the ANOVA, we confirmed there was no violation of the sphericity assumption using Mauchly's test of sphericity. Holm-Bonferroni was used as post-hoc test when significant differences were detected. Values are provided as the mean ± standard deviation (SD). Significance was set at $p < 0.05$.All statistical tests were performed using the software package SPSS v.18.0 (SPSS Inc., Chicago, IL, USA).

Results

Sprint Performance Variables

The effects of placebo and BJ on the 30-s all-out sprint test are shown in Table 1. Compared to placebo, BJ supplementation increased W_{peak} (~3.8%; $p = 0.049$) and W_{mean} (~4.0%; $p = 0.023$), while reduced timetoW_{peak} (~18%; $p = 0.003$). In 12 of the 15 participants, $W_{peak}$was higher after BJ administration

compared to the placebo condition (Figure 2). In contrast, no significant differences were observed in W_{min} (~4.4%; $p = 0.064$) or FI (~0.22%; $p = 0.914$).

Table 1. Effects of placebo or BJ intake on performance at a 30-s sprint (Wingate) test.

Variable	Placebo	BJ	*p*-Value
W_{peak} (W)	848 ± 134	881 ± 135	0.049
Time to W_{peak} (s)	8.9 ± 1.4	7.3 ± 0.9	0.003
W_{mean} (W)	641 ± 91	666 ± 100	0.023
W_{min} (W)	453 ± 64	472 ± 72	0.064
Fatigue index (FI) (%)	46 ± 8	46 ± 7	0.914

Values are means ± standard deviation. BJ, beetroot juice.

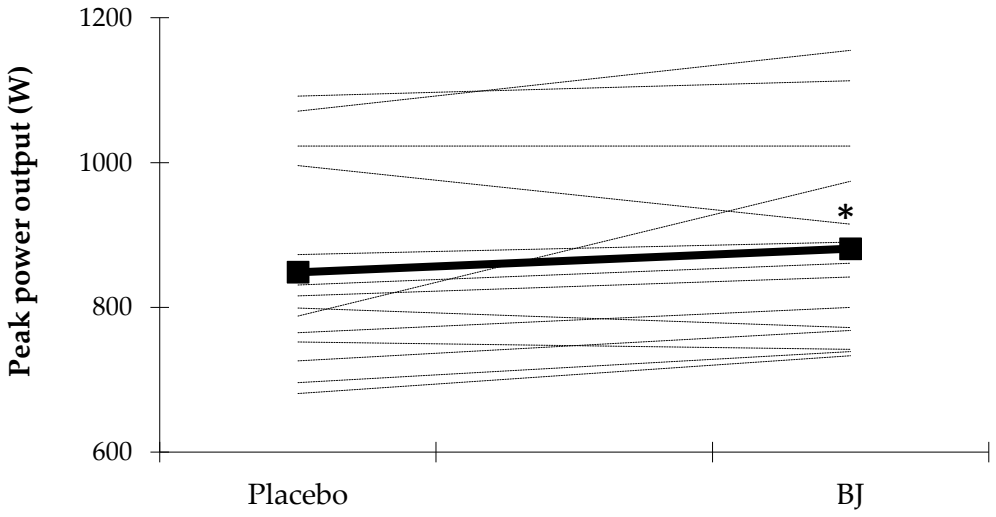

Figure 2. Effects of placebo and BJ intake on W_{peak} after sprint exercise. Means and individual values are shown as a bold ordotted line respectively.* $p< 0.05$ compared to placebo.BJ, beetroot juice.

Values of W_{mean} were recorded in 10 and 15 s intervals. Figure 3 displays W_{mean} values in 15 s intervals ($W_{0–15s}$ and $W_{15–30s}$). An increased W_{mean} was

observed after BJ intake compared to placebo during the first 15s of the sprint (placebo vs. BJ, 709 ± 113 vs. 740 ± 122 $W_{0–15s}$; p = 0.017), while no significant differences were recorded during the last 15s (placebo vs. BJ, 574 ± 80 vs. 593 ± 87 $W_{15–30s}$; p = 0.173).

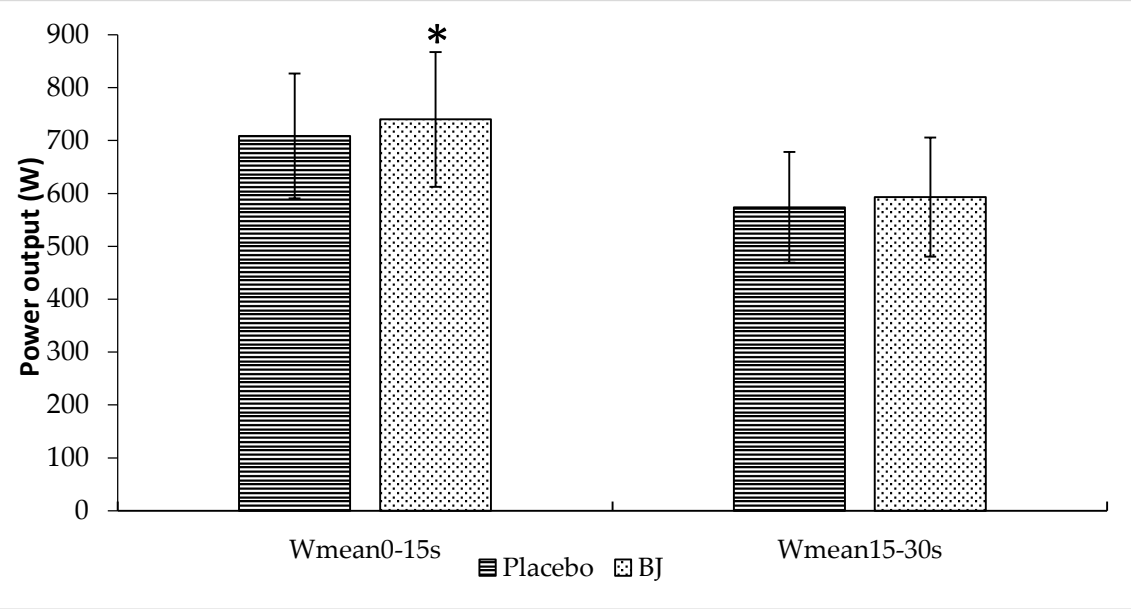

Figure 3. Effects of placebo and BJ intake on W_{mean}values recorded over 15 s intervals (A, $W_{0–15s}$; B, $W_{15–30s}$) after the sprint.* p< 0.05 compared to placebo.

Figure 4. provides W_{mean} values in 10 s intervals ($W_{0–10s}$, $W_{10–20s}$ and $W_{20–30s}$). Compared to placebo, a significant increase in $W_{mean}$was observed after BJ intake during the first 10s interval (placebo vs. BJ, 683 ± 118 vs. 717 ± 127 $W_{0–10s}$; 5.0%,p = 0.043), while no significant differences emerged for the intervals10–20s (placebo vs. BJ, 712 ± 105 vs. 735 ± 113 $W_{10–20s}$; 3.2%, p = 0.078) or 20–30 s (placebo vs. BJ, 529 ± 73 vs. 548 ± 79 $W_{20–30s}$; 3.6%, p = 0.30).

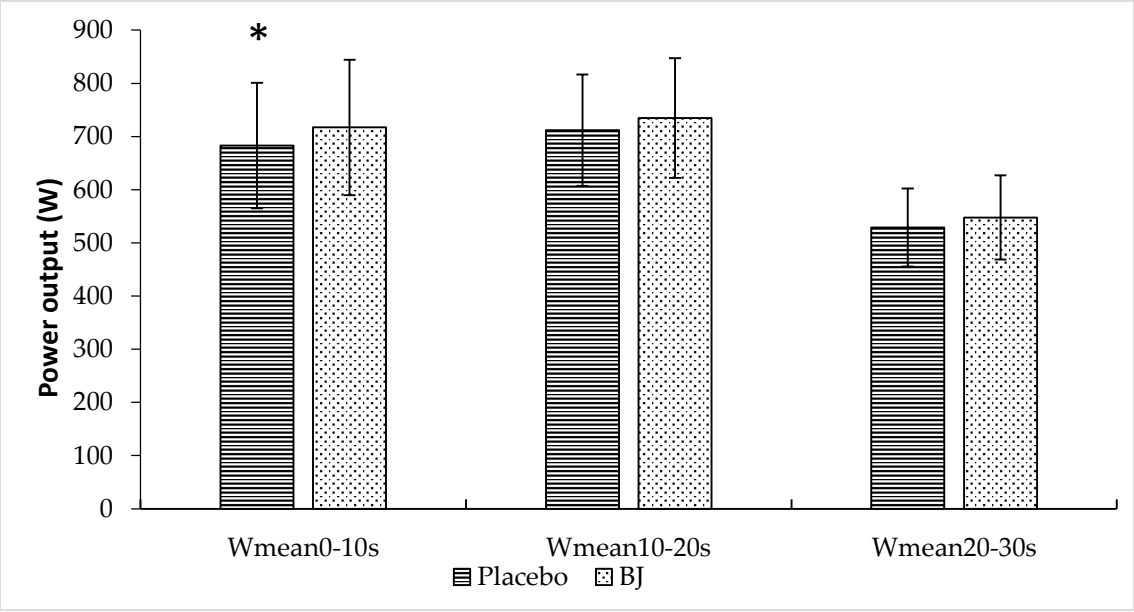

Figure 4. Effects of placebo and BJ intake on W_{mean}values recorded over 10 s intervals ($W_{mean0–10s}$, $W_{mean10–20s}$ and $W_{mean20–30s}$) after the sprint.Values are means ± standard deviation. * p< 0.05 compared to placebo.

Neuromuscular Fatigue and Blood Lactate Concentrations

The effects of placebo and BJ intake on neuromuscular fatigue measured through the CMJ test are shown in Table 2. The 30-s all-out Wingate test led to significant reductions in CMJ_{height}, CMJ_{Wpeak} and CMJ_{Wmean} (ANOVA time effect, p< 0.001). Compared to Pre, a significant decrease was observed at Post and Post-3 in CMJ_{height} (Pre vs. Post, ~38%; Pre vs. Post-3, ~19%; p< 0.001), CMJ_{Wpeak} (Pre vs. Post, ~28%; Pre vs. Post-3, ~10%; p< 0.001) and CMJ_{Wmean} (Pre vs. Post, ~21%; Pre vs. Post-3, ~14%; p< 0.001); while a significant increase was observed for Post-3 compared to Post in all variables (~24% CMJ_{height}, ~22% CMJ_{Wpeak}, ~21% CMJ_{Wmean}; p< 0.001). No supplementation or interaction effects (supplement × time) were observed.

Figure 5. illustrates the blood lactate values recorded after the sprint test. Blood lactate concentration was significantly higher after the 30-s all-out Wingate test (ANOVA time effect, p< 0.001). Compared to Pre (placebo, 1.47 ± 0.71 mmol/L; BJ, 1.47 ± 0.35 mmol/L), blood lactate was significantly higher at the time

points Post-0.5 (placebo, 13.86 ± 3.37 mmol/L; BJ, 14.49 ± 3.27 mmol/L; p< 0.001) and Post-3.5 (placebo, 15.20 ± 2.62 mmol/L; BJ, 14.84 ± 2.32 mmol/L; p< 0.001). No supplementation (ANOVA supplementation effect, p = 0.858) or interaction effects (ANOVA supplement × time effect, p = 0.719) were detected.

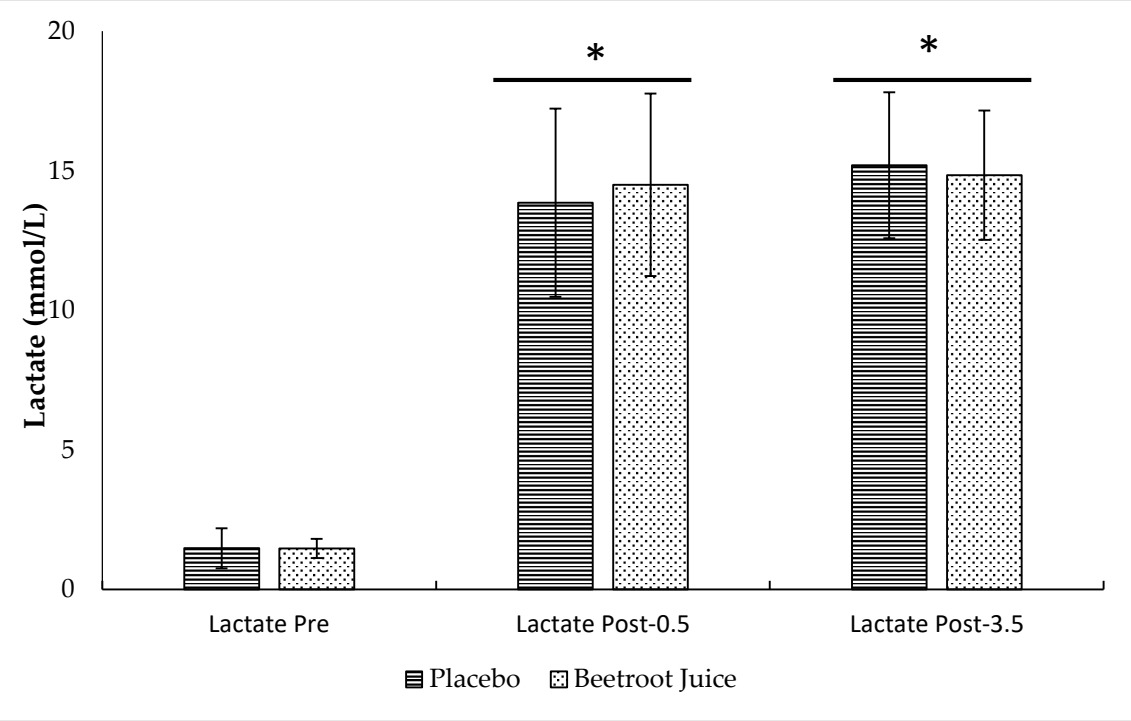

Figure 5. Blood lactate concentrations recordedafter the sprint for the placebo and BJconditions. Values are means ± standard deviation. * p< 0.05 compared to Pre.Pre, before sprint exercise; Post-0.5, 0.5 min post-exercise; Post-3.5, 3.5 min post-exercise.

Table 2. Effects of placebo or BJ intake in a neuromuscular fatigue (CMJ) after a 30-s all-out Wingate test.

Variable	Placebo			BJ			Suppl.	Time	Suppl. × Time
	Pre	Post	Post-3	Pre	Post	Post-3			
CMJ_{height} (cm)	30.8±4.6	19.5±5.1 [a]	25.0±4.3 [a,b]	31.5±3.4	19.0±4.2 [a]	25.3±4.2 [a,b]	0.863	<0.001	0.864
CMJ_{Wpeak} (W)	50.5±4.7	36.9±5.9 [a]	45.2±4.6 [a,b]	51.1±3.6	36.6±4.9 [a]	44.7±4.5 [a,b]	0.947	<0.001	0.850
CMJ_{Wmean} (W)	27.3±3.6	20.0±4.2 [a]	24.0±3.8 [a,b]	27.9±3.3	19.6±3.5 [a]	23.8±3.6 [a,b]	0.994	<0.001	0.850

Values are means ± standard deviation. [a]p< 0.05 compared to Pre; [b]p< 0.05 compared to Post. Pre, before sprint exercise; Post, post-exercise; Post-3, 3 min post-exercise.

Discussion

The findings of our study indicate that BJ supplementation enhances peak and mean power output, particularly during the first half of a 30-s all-out sprint test, reducing the time taken to reach peak power output. Despite this improved sprint performance, neuromuscular fatigue caused by this exercise mode was similar after the intake ofBJor placebo. These observations suggest that NO_3^--rich supplements enhance sprint performance without producing cumulative impacts on fatigue levels.

NO_3^-supplementation has been linked to an increase in W_{peak} generated by leg extension in an isokinetic machine at several angular velocities (from 0 to 6.28 rad/s) in healthy subjects (~5–6%) [2,31] and patients with heart disease (~12%) [32]. There are two reports in the literature of investigations examining the effects of an acute dose of BJ on a 30-s all-out Wingate test [19,23]. In the study by

Domínguez et al. [20], a significant increase in $W_{peak}$was observed (~6%) while Rimer et al. [23] observed no such effect. It should be mentioned that in the study by Rimer's group [23], the 30-s Wingate test was performed after 4 series of 3–4 s all-out sprint trials and 5 min of passive rest; and despite the lack of difference in the Wingate test, the delta change in peak power output produced in the 3–4 sprints indicated anincrease of ~6.0% after BJ intake compared to placebo. In the present study, a similar increase in peak power output was observed after the 30-s all-out Wingate test (~4%) and this performance improvement seems to occur during the first 15 s of the sprint and hereafter decline. These data indicate that BJ supplementation may cause a transient ergogenic elevation of peak power output during the first few seconds of sprint exercise, and that this effect could be attenuated after several doses of BJ [6].

The use of an isokinetic or isoinertial cycle ergometer for the sprint test may be a confounding factor when examining the ergogenic effect of BJ supplementation [19,23]. In this study, we used an isoinertial cycle ergometer, which measures power output based on a variable pedaling rate at a fixed load (7.5% body mass) [20].In contrast,using an isokinetic cycle ergometer, thepedaling rate is predetermined [23]. Pedaling rate is strongly related to the angular velocity of the knee and hip, and can be used as an indicator of muscle contraction velocity [33] and type II motor unit recruitment [34]. An ergogenic effect of BJ intake has been observed not only in sprint exercise [20] but also in other tasks (e.g., leg extension) under elevated angular velocities [2,31,32]. Consistent with this idea, the present data revealed a greater effect of BJ on sprint performance (W_{peak} and timetoW_{peak}) for the higher angular velocities.

Animal studies have shown that NO increases acetylcholine activity, particularly in type II motor units, which amplify depolarization of the muscle fibers [35] whereas BJ supplementation induces the elevation of intracellular Ca^{2+} concentrations accompanied by calsequestrin 1 and dihydropyridine receptor upregulation in fast-twitch muscles [18]. Although these mechanisms have not yet been proven in humans, NO_3^- supplementation likely increases force production by inducing type II muscle fiber depolarization and increasing myoplasm Ca^{2+} concentrations facilitating muscle contraction [18,36] by

increasing the number of actin-myosin cross-bridges [37]. This improvement in muscle force production in response to BJ consumption has been detected as a higher rate of force development (RFD) [37] through increased peak power output, the time taken to reach that power output and a fasterreaction time [4].

In effect, Time$to W_{peak}$ and reaction time are key factors in sports performance, particularly in disciplines in which acceleration determines performance [38,39]. Here, BJ supplementation led to a pronounced reduction in Time$to W_{peak}$during a 30-s all-out Wingate test, coinciding with previous data in which the increase in W_{peak} was accompanied by a shorter time needed to reach W_{peak} [20]. Areduced Time$to W_{peak}$ was also found when a transient increase in W_{peak} was not detected after prolonged doses of BJ supplements and repeated sprint exercise [6]. In these two previous studies [6,20], the shortenedTime$to W_{peak}$ was lower (~0.7 and ~0.2 s, respectively) than the difference observed here (~1.6 s). The greater improvement in Time$to W_{peak}$ reported here may be explained by a reduced level of anaerobic training of our subjects compared to participants of the studies by Dominguez et al. [20] and Jonvik et al. [6],who were well-trained in anaerobic disciplines.

Anaerobic pathways supply ~75% of energy requirements in a 30-s all-out sprint exercise [40,41]. During the first 6 s, ready to use sources of energy are needed to produce maximal peak power output in the shortest time possible.Accordingly, free ATP and PCr stores are critical during the initial part of a sprint [42]. At this time (first 5–10 s), a marked depletion in PCr stores occurs and this compromises power output coinciding with the time at which glycolysis attains its maximum rates [43]. Along with an increased force production capacity, BJ supplementation leads to the reduced ATP cost of muscle contraction [19,44] perhaps by reducing PCr degradation rates. The reduced ATP requirements of muscle contraction together with the maintenance of free ATP and PCr stores promoted by NO_3^- supplementation may give rise to a higher power output during a longer period of time coinciding with the increase in mean power output produced during the first 15s of the sprint after BJ intake.

Since BJ consumption led to elevated peak and mean power output during the first 15 s of the sprint, we could argue that the muscular fatigue that takes place during the last 15s and at the end of the sprint will be exaggerated.

The fatigue index calculated during the sprint indicated no differences between the supplements. In addition to the mentioned maintenance of anaerobic sources of energy production, the contribution of aerobic energy production increases during the last 15 s of a Wingate test [41,43]. Since NO_3^- supplementation is known to reduce the oxygen cost of ATP synthesis [45] and to preserve ATP and PCr stores [19], the lack of differences between supplements (placebo vs. BJ) may be explained by a higher capacity of NO_3^- to induce ATP store maintenance and thusreduce the cost of its synthesis by both aerobic and anaerobic sources.

Immediately after the sprint exercise, two CMJ jumps were performed at 30 s and 180 s. CMJ is a vertical jump test that assesses muscle contractile propertiesand neuromuscular performance (anaerobic power) of the lower-limbs [46,47]. Variables such as CMJ height and power have also been used as indicators of neuromuscular fatigue [48,49]. Some authors have argued that the CMJ testafter extenuating exercise [28] serves to assess muscle capacity to replenish ~50% of depleted PCr stores at 30-s post-exercise [50] and to recover almost completely depleted PCr stores at 180 s post-exercise [51]. Hence a pronounced reduction in CMJ performance (height and power) after 180s will reflect the diminishedPCr store replenishment capacity of muscle fibers affecting the stretch-shortening cycle and force production [52]. The present observations are in good agreement with prior findings in which an effect of time in reducing CMJ height and mean power output was seen after a 30-s all-out Wingate test [28–30]. The decrease in CMJ performance was more pronounced at 30 s (~30%) compared to 180 s post-exercise (~10%).However,no differences between supplementation conditions were observed.

In our study, BJ supplementation overall did not give rise to a greater fatigue index during the second half of the test or to neuromuscular fatigue as measured in CMJ tests, after the 30-s all-out sprint test. These results indicate that the

improved sprint performance induced by BJ as aNO_3^--rich supplementmay not be accompanied by more fatigue.

Limitations

Our study has several limitations**.**BJ is a NO_3^--rich supplement known to increasecirculating NO_2^-andNO levels [7].However,these levels were not measured beforethe intake by the participants of placeboorBJ.Further, the number of subjects recruited (*N* = 15), although appropriate for this type of study, limits the detection of small changes that could be the consequence of BJ administration. Finally, participants were not trained cyclistsand therefore the ergogenic effects produced by BJ cannot be directly transferred to this sports modality.On the up-side, however, the inclusion of resistance trained individuals was useful to explore the physiological effects of BJ supplementation on skeletal muscle power production and to examine fatigue induced by a sprint exercise to exhaustion.

Conclusions

In conclusion, BJ supplementation produced an ergogenic effect in a 30-s all-out Wingate test in terms of increasing W_{peak}, TimetoW_{peak} and W_{mean}, particularly during the first half of the sprint, without increasing muscular fatigue accumulation during or after this extenuating sprint exercise. These findings suggest that NO_3^--rich supplements could be a suitable strategy to improveperformance in sports modalities in which power and acceleration largely determine performance.

Author Contributions: R.D. and S.F.d.S. designed the experiment; P.J. and P.V.-H. recruited the subjects and hosted the informative session; P.V.-H. and L.G.G.-R. checked that subjects followed the diet guidelines and the timing of supplement ingestion. R.D., E.C., P.J., P.V.-H. and L.G.G.-R. performed the experiments; E.C., A.P.-L. and S.F.d.S. analyzed the data; R.D. and S.F.d.S.

conducted the statistical analysis; and R.D., E.C., P.J., P.V.-H., L.G.G.-R. A.P.-L. and S.F.d.S. wrote the manuscript.

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5. DISCUSIÓN

Para un mejor entendimiento dividiremos la discusión en dos partes:

- *Esfuerzos prolongados prioritariamente aeróbicos.*
- *Esfuerzos de alta intensidad predominantemente anaeróbicos.*

5.1 Esfuerzos prolongados prioritariamente aeróbicos.

Tras la primera intervención experimental, se evaluaron los efectos de la suplementación aguda de ZR sobre el rendimiento cardiorrespiratorio en triatletas entrenados. El principal hallazgo de este estudio fue que no se observaron ningún tipo de mejoras en las variables cardiorrespiratorias estudiadas, tomando una dosis de 6,5 mmol de NO_3^-. Sin embargo, el tiempo en el VT2 se incrementó un minuto, a pesar de que esta mejora no fue significativa. Contrariamente a nuestra hipótesis de estudio, no se detectó que la dosis aguda utilizada de ZR mejorara las respuestas cardioventilatorias, la economía / eficiencia mecánica, el componente lento del VO_2, el uso de sustratos de energía o el rendimiento en estos atletas. A pesar de que los atletas nacionales estaban menos entrenados que los atletas internacionales, no se observó ningún efecto positivo de la suplementación con ZR en atletas tanto internacionales como nacionales.

Nuestros resultados confirmaron los obtenidos en otros estudios, en los cuales no se hallaron mejoras en el tiempo hasta la extenuación (80), ni en el rendimiento de las variables cardioventilatorias (frecuencia cardíaca, ventilación, equivalente ventilatorio de oxígeno, tasa de intercambio respiratorio) a intensidades submáximas (58). Nuestros hallazgos indican que, probablemente, dosis más elevadas son necesarias (> 8,4 mmol) para mejorar el rendimiento cardiorrespiratorio en triatletas entrenados.

Los datos observados a intensidad moderada de VT1 están refrendados por los resultados de otras investigaciones (58,80) en las que no hubo mejoras en las respuestas cardioventilatorias al ejercicio submáximo después de la suplementación con NO_3^-.

Al no observarse ningún efecto positivo, se puede sugerir que la dosis no fue la adecuada para incrementar el rendimiento cardiorrespiratorio a intensidad de VT1. Estudios previos han demostrado que una mayor dosis de suplementación de ZR (~ 8,4 mmol y ~ 16,8 mmol de NO_3^-) causó una mayor reducción en la presión arterial sistólica y la presión arterial media a una intensidad de ejercicio moderada que las dosis más bajas (~ 4,2 mmol de NO_3^-) en adultos sanos (81). En este estudio, el VO_2 durante una intensidad moderada del ejercicio, se redujo significativamente después de la ingesta de 16,8 mmol de NO_3^-, con una tendencia a ser menor después de la ingesta de 8,4 mmol de NO_3^-, y no fue afectado por 4,2 mmol de NO_3^-. Las dosis más altas de suplementos de ZR (2 x 70 ml de dosis por día, ~ 6,2 mmol de NO_3^- por 70 ml) antes y durante el ejercicio prolongado de intensidad moderada podrían ser necesarias para atenuar el aumento progresivo del VO_2 y reducir el agotamiento del glucógeno muscular (79), mejorando la eficiencia mecánica durante una prueba de carga constante prolongada a intensidad VT1. Además, la declaración de consenso del Comité Olímpico Internacional, concluye que los períodos más largos (> 3 días) de NO_3^- la suplementación con ZR podría aumentar el rendimiento deportivo en atletas altamente entrenados (84).

Se sabe que los individuos más expertos presentan un mejor control vascular, caracterizado por una mayor actividad y presencia de la enzima endotelial óxido nítrico sintasa (eNOS), responsable de la producción endógena de NO (104). Por lo tanto,

cualquier aumento en la actividad de eNOS podría reducir la disponibilidad de NO disminuyendo los posibles efectos de ZR. Este razonamiento podría explicar, al menos en parte, los resultados obtenidos para el VT1, ya que se trata de una intensidad de ejercicio de baja a moderada donde las exigencias de VO2 son bajas, después de la cual se produce un primer cambio en la ventilación y en las concentraciones de lactato en sangre, por encima del cual está involucrado parcialmente el metabolismo energético anaeróbico (105).

Las concentraciones de lactato en nuestros atletas en VT1 fueron inferiores a 2 mmol que indica un estado predominantemente aeróbico. Durante esta etapa metabólica, tal intensidad de ejercicio puede mantenerse durante un largo período de tiempo sin cambios marcados en las concentraciones de lactato en la sangre (46,47). Por lo tanto, es menos probable que un atleta entrenado experimente baja oxigenación muscular, lo que aumenta la acidosis muscular y genera una reducción de nitrato a una tasa de trabajo determinada (46). Sospechamos que no hubo un efecto positivo de ZR, ya que los sujetos más entrenados podrían mostrar una reducción en la absorción de oxígeno debido a una disminución en la energía aeróbica requerida o en la energía muscular utilizada en los esfuerzos de ejercicio moderado.

Aunque no se detectaron mejoras significativas estadísticas en la duración de la prueba tras la suplementación con ZR, estos hallazgos apuntan a un posible efecto ergogénico del ZR en el rendimiento cardiorrespiratorio de atletas de deportes de resistencia altamente entrenados. Se debe considerar que para aumentar la posibilidad de ganar, un atleta de deportes de resistencia de alto nivel debe lograr una ganancia en el tiempo total de al menos 0,6% (106). Por ejemplo, la variación entre el duodécimo y el primer lugar

en la final de carrera de 10,000 metros masculinos en los Juegos Olímpicos de Londres 2012 fue solo del 0,66% (107). Si los triatletas de este estudio mejoraron de media aproximadamente un minuto, es un tiempo más que considerable para incrementar el rendimiento. Disminuir el tiempo en una prueba de duración aproximada de 45 minutos en un minuto no está al alcance de muchos atletas. Por ello, creemos que el ZR tuvo una incidencia positiva en los mecanismos fisiológicos que conducen a una disminución en el tiempo de la prueba. Sin embargo, faltan aún por esclarecer que procesos fisiológicos específicos subyacen de la suplementación aguda con ZR.

Intentando dilucidar estos mecanismos, se analizó la eficiencia y la economía mecánica durante la prueba. Nuestros cálculos de eficiencia mecánica se dirigieron a evaluar los efectos de la alcalinización de la sangre en las pérdidas graduales en la eficiencia muscular como el mejor indicador del fenómeno llamado componente lento del VO_2 (108). Con este protocolo induciendo un cambio en el metabolismo de VT1 a VT2, intentamos examinar los efectos del ZR después de promover un cambio en la cinética del VO_2 (componente lento).

Este cambio es similar al observado en el inicio del ejercicio de alta intensidad, lo que da lugar a un aumento de la liberación de oxígeno en el músculo, un incremento de la actividad de las enzimas metabólicas oxidativas, a la disponibilidad de sustratos de carbono y patrones anormales de reclutamiento de unidades motoras (109,110). Tampoco se observó ningún cambio en el componente lento del VO_2 y en la eficiencia/economía en nuestra investigación entre ambas condiciones experimentales.

Hay algunas limitaciones en este estudio que deben ser consideradas. Investigaciones anteriores indican que el NO_2^- en plasma de los participantes debería aumentar para mostrar un efecto ergogénico; sin embargo, las concentraciones de NO_2^- y NO_3^- en plasma no se midieron en nuestro estudio. Parece que las dosis cercanas o mayores a 8,4 mmol son más adecuadas para determinar los efectos positivos de la suplementación con BJ sobre el rendimiento de resistencia en triatletas bien entrenados.

El pequeño tamaño de la muestra en este estudio debe tenerse en cuenta al extraer conclusiones de los datos, especialmente al observar la tendencia estadística a la mejora en el tiempo a la intensidad de VT2. Los cambios mínimos en el rendimiento de la resistencia generalmente se observan en triatletas bien entrenados, por lo tanto, se deben requerir tamaños de muestra grandes para detectar los cambios significativos producidos por la suplementación con ZR en el rendimiento cardioventilatorio.

5.2 Esfuerzos de alta intensidad predominantemente anaeróbicos.

Una vez terminada la revisión sobre esfuerzos anaeróbicos y las dos intervenciones experimentales en esfuerzos de alta intensidad, podemos hacer una valoración mas amplia sobre el efecto ergogénico del ZR en esfuerzos predominantemente anaeróbicos. En primer lugar, encontramos muy poca literatura científica donde estudien los efectos del ZR en deportes de alta intensidad. En la revisión realizada y tras los criterios de exclusión, solo 9 artículos reunían los requisitos pautados, observando que solo 5 de ellos (70,76,77,78,99), utilizaban de forma aguda esta ayuda ergonutricional. En tres había mejoras (76,77,111) ya sean en pruebas de campo (realizando un mayor número de series hasta el agotamiento) y potencia en los test en cicloergómetro,

De los 5 artículos seleccionados que tomaban ZR de forma aguda, en 2 de ellos no había ningún tipo de mejora significativa (70,78). Al igual que en la revisión realizada por Domínguez y cols. (103), donde también se observó controversia sobre este tipo de suplementación en esfuerzos de resistencia de menor intensidad. Este resultado hace pensar la importancia de seguir profundizando en el tema, y realmente ver los efectos en test o pruebas específicas.

La mayoría de los artículos encontrados durante la revisión, utilizan dosis entre 4,9 y 9 mmol, siendo entre 2-3 horas antes de la actividad las pautas de recomendación para la ingesta de ZR. Además, los efectos del ZR podrían potenciarse con la cafeína (112-115).

Aunque en esta tesis doctoral nos centremos en la suplementación de forma aguda, durante esta revisión observamos varias intervenciones donde el ZR se suministraba de forma crónica, con unos periodos comprendidos entre cinco a siete días. Mosher y cols. (111) estudiaron el efecto del ZR durante una sesión de entrenamiento con resistencias (resistance training) durante 6 días, con una dosis de 6,5 mmol de NO_3^-. Se analizaron el número de repeticiones realizadas sobre un total de tres series con una carga correspondiente al 60% de 1 RM en el ejercicio de press de banca. En dicha investigación se comprobó que la suplementación aumentó el número de repeticiones en las tres series de ejercicio realizadas, mejorando el trabajo total realizado durante la sesión un 18,9%.

En otro estudio se valoró la suplementación de ZR de forma crónica, con objeto de comprobar la capacidad de repetir esfuerzos de alta intensidad durante un partido en deportes colectivos. Thompson et al. (101) administraron a un grupo de deportistas un

protocolo de suplementación con ZR (12,8 mmol de NO_3^-) durante 7 días. El test empleado evaluaba la capacidad de repetir esfuerzos de alta intensidad y consistió en la realización de 2 bloques de 5 series de 6 segundos de sprints en cicloergómetro, intercalados con 14 segundos de recuperación activa a la mitad y finalizando con un test de simulación de los deportes colectivos de 2 x 40 minutos de duración. El resultado de la investigación fue el aumento del trabajo total realizado en la sesión de un 3,5%, si bien, las mejoras fueron superiores al finalizar la primera mitad (mitad del partido de simulación), que durante la segunda mitad. En otro estudio, los protocolos que incluían esfuerzos de 30 y 60 segundos de trabajo, sin embargo, la suplementación con ZR no mejoró ninguna variable relacionada con el rendimiento deportivo (102). Estos protocolos que incluían períodos de trabajo de mayor duración son esfuerzos que implican mayoritariamente al metabolismo glucolítico y, en menor medida al sistema de los fosfágenos de alta energía.

En otra investigación (116) con jugadores de fútbol, se comprobó los efectos de la suplementación crónica (5 días) con ZR (8,4 mmol de NO_3^-), en esta ocasión sobre el rendimiento en test repetidos de alta intensidad. Los investigadores quisieron comprobar el efecto de la suplementación ante distintos tipos de protocolos de ejercicio, de este modo, los sujetos realizaron una sesión consistente en 24 series de 6 segundos con 24 segundos de recuperación, una segunda sesión en la que se realizaron 2 series de 30 segundos con 2 minutos de recuperación y una tercera sesión consistente en 6 series de 60 segundos de trabajo y 60 segundos de recuperación.

Al igual que Thompson y cols. (101), Krustrup y cols. (116) escogieron períodos de 6 segundos de trabajo en la primera de las sesiones, si bien, los períodos de recuperación fueron sensiblemente superiores (24 vs 14 segundos). Los resultados fueron similares, observando una mejora en la potencia media de las series en el total de la sesión de ~7%. Sin embargo, las mejoras comprobadas en el test de 24 x 6-24 no fue extrapolable a los otros dos test, donde no se observó ninguna diferencia significativa.

Estas mejoras probablemente estén asociadas al reclutamiento de unidades motoras tipo II (116-117), más potentes, aunque con una menor resistencia a la fatiga (118). La menor resistencia a la fatiga se debe por una irrigación y concentraciones de mioglobina más pobres en las unidades motoras tipo II en relación a las unidades motoras tipo I. Las unidades motoras tipo II, por el contrario, están diseñadas para favorecer los procesos no oxidativos, habiéndose demostrado una mayor capacidad de almacenamiento de creatina (119), favoreciendo el metabolismo de la fosfocreatina (120), y de proteínas con efecto tampón a nivel intracelular, como es el caso de la carnosina (121), favoreciendo el metabolismo glucolítico. Estudios en animales han comprobado que las mejoras en la perfusión como respuesta a la suplementación con NO_3^- es superior en las unidades motoras tipo II con respecto a las unidades motrices tipo I (59).

Siguiendo con las valoraciones encontradas en los estudios con ZR en esfuerzos de alta intensidad, durante nuestra segunda y tercera intervención experimental se encontraron resultados semejantes, en ambos se evaluó los efectos del ZR en el rendimiento anaeróbico en sujetos entrenados, mediante un test de Wingate y en la última intervención además se valoró la fatiga mecánica medida con un salto en contramovimiento (CMJ).

En ambas investigaciones mejoraron los niveles de potencia. En la segunda intervención fueron de un +5,4 % así como una tendencia significativa (p = 0.055) en el tiempo hasta alcanzar el pico de potencia, y niveles más altos en la potencia media. En la tercera intervención se encontraron diferencias estadísticamente significativas en los valores de potencia pico (+3,8%), tiempo en alcanzar la potencia pico (-17,98%) y potencia media (+3,96%).

A diferencia del estudio realizado por Clifford y cols. (78), podemos sugerir que la suplementación con ZR podría preservar la estructura del músculo durante esfuerzos de alta intensidad. Si bien, otra posible explicación podría residir en que el efecto vasodilatador del ZR (49), podría haber facilitado los procesos de regeneración muscular que tienen lugar en la recuperación temprana.

Si atendemos a los factores que influyen en la producción de potencia, es destacable la influencia del tipo de unidad motora reclutada, al tener las unidades motoras tipo II una mayor velocidad y fuerza de contracción muscular (122,123). En este sentido, se ha comprobado que la mejora de la suplementación con ZR sobre el pico de fuerza en una contracción isométrica es específica de las unidades motoras tipo II (124). Investigaciones en animales, también, han demostrado que los efectos de la suplementación con ZR sobre la perfusión sanguínea (61) y la producción de fuerza (125) es específica de las unidades motoras tipo II. De hecho, estudios en animales han demostrado que el NO aumenta la acción de la acetilcolina exclusivamente en las unidades motoras tipo II (126).

Mejoras en la acción de la acetilcolina pueden amplificar la despolarización de la motoneurona (126). El ZR aumenta la expresión de la calsecuestrina (125), incrementando la liberación de calcio desde el retículo sarcoplasmático al sarcoplasma de la fibra muscular (127), dónde se fijará a la tropomiosina y a la troponina para facilitar el entrecruzamiento de la actina y la miosina. El aumento en la sucesión de potenciales de acción y de la presencia de calcio en el sarcoplasma podría aumentar la producción de potenciales de acción en ráfaga, aumentando la máxima producción de potencia, tal y como ha sido comprobado al analizar la actividad electromiográfica en esfuerzos de máxima intensidad (128).

Además de los efectos de la suplementación con ZR sobre la producción de fuerza en las unidades motoras tipo II, distintas investigaciones en animales han informado que el ZR reduce el coste de ATP durante el esfuerzo (59), observando una menor degradación de PCr tanto en ejercicio de baja como de alta intensidad (129). Una disminución del coste de PCr durante el esfuerzo de máxima intensidad (130) retrasaría la depleción de las reservas de PCr (57,131). Dado que la PCr es fundamental para los esfuerzos de alta intensidad (70), un enlentecimiento en la depleción de dicho sustrato durante la ejecución del test de Wingate hubiese facilitado el mantenimiento de unos mayores picos de potencia durante la primera parte del test, explicando las mejoras significativas en ambas intervenciones.

Los efectos reportados en estas investigaciones refuerzan los encontrados (100) sobre un mismo test (test de Wingate en cicloergómetro inercial) en una población de las mismas características y tipo de entrenamiento (deportistas de Crossfit), tras comprobar los efectos de la suplementación con sales de nitrato. En dicha investigación, se comprobó que la suplementación con 8 mmol de nitrato potásico provocó mejoras del 6,6% (6% en nuestra investigación) en el pico de potencia máxima. Aunque no se observó una mejora significativa en la potencia media desarrollada durante el test, se detectó una tendencia a la significación estadística (p=0.08). Sin embargo, la ausencia de comparativa en la producción de potencia durante intervalos del test hace imposible comparar si en dicho estudio, también, hubo una mejora significativa durante los 15 primeros segundos del test. Otros estudios refuerzan los resultados encontrados en potencia (77,132), determinando que estas mejoras podrían estar relacionadas con la mayor activación de unidades motoras tipo II (124,126,128).

El aumento de las concentraciones de lactato sanguíneo encontrado en ambas intervenciones tras la suplementación con ZR coincide con el reportado en otras investigaciones (102,107). De este modo, Wylie y cols. (102) observaron un aumento de las concentraciones de lactato sanguíneo junto a un aumento del rendimiento en un protocolo consistente en 24 series de 6 segundos con 24 segundos de recuperación en cicloergómetro. Este aumento en la producción de potencia tras la suplementación con ZR se debe a un aumento del rendimiento en las unidades motoras tipo II y a que dicho tipo de unidad motora se caracteriza por una mayor dependencia del metabolismo glucolítico, aumentando en mayor medida las concentraciones de lactato tras el esfuerzo (133).

El flujo del lactato al exterior de la célula depende de la capilarización (134) y, en este sentido, es posible que el aumento de la perfusión de las unidades motoras tipo II tras la suplementación con ZR (59), podría haber aumentado las concentraciones de lactato sanguíneo.

Al comparar posibles diferencias metodológicas en nuestras investigaciones y la realizada por Rimer y cols. (77), en la que no se encontró ningún efecto ergogénico de la suplementación con ZR sobre potencia máxima, nos encontramos que ellos emplearon un cicloergómetro isocinético. Nosotros en cambio, al igual que las dos investigaciones previas que han reportado mejoras significativas de la suplementación con ZR sobre potencia pico (W_{pico}) (77,132), hemos empleado un cicloergómetro inercial. La producción de potencia en ambos tipos de ergómetros difiere, de este modo, mientras los cicloergómetros isocinéticos valoran la producción de potencia como los cambios en la aplicación de fuerza a una cadencia de pedaleo previamente determinada, los cicloergómetros inerciales valoran la potencia en base a los cambios de la cadencia de pedaleo ante una carga constante (generalmente empleando un porcentaje de masa corporal) (132) . Debido a que la cadencia de pedaleo ha demostrado tener una correlación muy alta con la velocidad angular de la rodilla y la cadera, ésta se utiliza como un indicador de la velocidad de acortamiento de la musculatura fijada a ambas articulaciones (135). Además, se sabe que el aumento de la cadencia de pedaleo hace que haya un mayor reclutamiento de unidades motoras tipo II (136).

Por tanto, el efecto sobre la amplificación de la despolarización de las unidades motoras tipo II mediadas por la acción del NO, junto al aumento de la biodisponibilidad de calcio (125), que aumenta el número de puentes cruzados, da lugar a un aumento de la fuerza

desarrollada (137) que se traduce en una mejora de la potencia máxima, así como en el tiempo en alcanzar la misma. Hay que indicar que mejoras en el tiempo hasta alcanzar la potencia máxima es uno de los factores de rendimiento de todas aquellas modalidades deportivas en las que la producción de máximos niveles de potencia y la aceleración son factores de rendimiento, como es el caso de los deportes colectivos (138), las pruebas de velocidad en atletismo, las pruebas de velocidad en ciclismo en velódromo o el patinaje de velocidad (139). Por tanto, la suplementación con ZR podría ser adecuada para incrementar el rendimiento en acciones musculares específicas de diversas modalidades deportivas donde el desarrollo de los niveles de potencia máxima sea determinante. Al respecto, más estudios son necesarios para determinar el posible efecto ergogénico de la suplementación con ZR en diferentes modalidades deportivas. No hubo diferencias significativas en la fatiga mecánica entre ambas condiciones experimentales. El ZR no tiene efectos sobre la fatiga mecánica medida mediante un CMJ, corroborando los datos propuestos por las pérdidas de capacidad de salto tras una sesión de entrenamiento (47,111).

Respecto a las limitaciones de estos estudios, encontramos en que estos deportistas al practicar esfuerzos de alta intensidad, tienen más desarrolladas las fibras tipo II, ya que principalmente son las responsables durante las realización de esfuerzos de alta intensidad, por este motivo y al no existir ningún tipo de estudios en personas donde se extraigan algún tipo de biomarcadores fisiológico (cortisol, testosterona, glucógeno, biopsias musculares fibras tipo II, etc. tras la suminitración de ZR), basamos nuestros nuestras evidencias científicas en artículos donde si se han tomado biomarcadores sobre el efecto del ZR en esfuerzos de alta intensidad y su relación con la mayor reclutamiento en este tipo de fibras pero en animales, en próximas investigaciones, hace necesaria la

inclusión de este tipo de medidas en humanos para conocer mejor los cambios que tienen lugar.

6. CONCLUSIONES

Tras los estudios realizados anteriores podemos concluir:

- Existe controversia sobre los efectos positivos del ZR en el rendimiento cardiorrespiratorio en sujetos entrenados. Nuestros hallazgos no determinan una mejora en las variables cardiorrespiratorias, en el componente lento de oxígeno y en la eficiencia/economía producidas en respuesta a la suplementación aguda de ZR.

- Todavía se tienen que dilucidar los posibles efectos ergogénicos de ZR en atletas altamente entrenados en deportes de resistencia aeróbica prolongados. Sin embargo, las mejoras leves (no significativas) observadas en las variables de rendimiento, como la duración de la prueba (~1 minuto) o el mantenimiento de la intensidad de trabajo ante una carga dada, plantean numerosos interrogantes, ya que los mecanismos mecánicos y fisiológicos analizados hasta ahora siguen siendo poco conocidos.

- Existen pequeñas mejoras, no significativas en deportes de resistencia cardioventilatoria, siendo las dosis mayores a 8 mmol de NO_3^- necesarias para confirmar un efecto positivo del ZR en el rendimiento cardiorrespiratorio en sujetos entrenados.

- La suplementación con ZR tiene un efecto ergogénico sobre el rendimiento en un esfuerzo de alta intensidad, como el test de Wingate, mejorando W_{pico}, Tiempo W_{pico} y W_{med}. Estas mejoras podrían dar pie a interpretar un incremento en el rendimiento en modalidades de alta intensidad, así como de aquellas que requieran aplicar máximos niveles de potencia o aceleración.

- La suplementación con ZR tiene un efecto ergogénico sobre la producción de potencia máxima, así como una tendencia en el tiempo en alcanzar ésta, la potencia durante los 15 primeros segundos de un test de Wingate en un cicloergómetro inercial.

- La suplementación con ZR incrementa las concentraciones de lactato post-ejercicio. Probablemente, dichos efectos sean debidos a las posibles mejoras específicas sobre la producción de fuerza y perfusión de las unidades motoras tipo II.

- La suplementación con ZR no tiene efecto ergogénico sobre la fatiga mecánica durante un esfuerzo de alta intensidad.

- Existe controversia sobre los efectos positivos del ZR en esfuerzos de alta intensidad.

- Las recomendaciones para la ingesta aguda de ZR para esfuerzos de alta intensidad se sitúan entre 2-3 horas antes de actividad, siendo la dosis recomendada entre 6,4 y 12 mmol en función de la condición física de los deportistas.

- Nuestros resultados sugieren la necesidad de analizar las respuestas positivas individuales a esta forma de suplementación en atletas bien entrenados.

Hacia donde enfocar el futuro

- Debido a que se ha propuesto que la suplementación con ZR mejora la capacidad de resíntesis de PCr, durante los breves períodos de recuperación que se incluyen en los protocolos intermitentes de alta intensidad, podría acelerarse debido a una

potenciación del metabolismo oxidativo durante la recuperación, retrasando la depleción de dicho sustrato energético; con lo que futuras investigaciones deberían comprobar, mediante biopsia muscular, las concentraciones de PCr durante la realización de protocolos repetidos de esfuerzos de alta intensidad.

- Con objeto de comprobar el mecanismo por el que la suplementación con ZR disminuye la fatiga mecánica y la recuperación de la misma, futuras investigaciones deberían comprobar el efecto de la ingesta de NO_3^- sobre biomarcadores de la inflamación y daño muscular.

- Debido a los resultados de una investigación que ha comprobado un efecto ergolítico de la suplementación aguda administrada 120-180 minutos antes, futuras investigaciones deberán comprobar cuál es el timing adecuado, con objeto de que los deportistas de este tipo de modalidades deportivas puedan optimizar el potencial ergogénico de esta suplementación.

- Por último, debido a que la suplementación con ZR podría tener un esfuerzo positivo ante esfuerzos de alta intensidad, futuras investigaciones deberán comprobar la interacción de la suplementación con ZR con otros suplementos que han demostrado tener un efecto ergogénico ante este tipo de esfuerzos como la cafeína, creatina, β-alanina y bicarbonato sódico.

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8. ANEXOS

Póster: X simposio internacional de actualizaciones en entrenamiento de la fuerza

POLITÉCNICA INEF NSCA® SPAIN NATIONAL STRENGTH AND CONDITIONING ASSOCIATION

Madrid, España

15 y 16 de Diciembre de 2017

X SIMPOSIO INTERNACIONAL DE ACTUALIZACIONES EN ENTRENAMIENTO DE LA FUERZA

Se certifica que

Eduardo Cuenca, Manuel Vicente Garnacho-Castaño, Pablo Veiga-Herreros, Felipe Santa Maria Damas, José Luis Maté-Muñoz, Raúl Domínguez han presentado el póster titulado **"Efectos de la suplementación con zumo de remolacha sobre el rendimiento en un esfuerzo de alta intensidad."** en el X Simposio Internacional de Actualizaciones en Entrenamiento de la Fuerza realizado en la **Facultad de Ciencias de la Actividad Física y del Deporte (INEF)** de la Universidad Politécnica de Madrid, entre los días 15 y 16 de diciembre de 2017.

Madrid, 16 de diciembre 2017.

Pedro J. Benito Peinado
Presidente del
Comité organizador

Ana B. Peinado Lozano
Presidenta del
Comité Científico

112 Número de certificados 6

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VII Jornadas Nacionales de Medicina del Deporte
El ejercicio: de la prevención al tratamiento
Zaragoza 24 y 25 noviembre 2017

D. PEDRO MANONELLES MARQUETA, en su calidad de presidente del Comité Organizador de las VII JORNADAS NACIONALES DE MEDICINA DEL DEPORTE, DE LA PREVENCIÓN AL TRATAMIENTO,

CERTIFICA que:

La comunicación científica: **Efectos de la suplementación con zumo de remolacha en la cinética del VO2 en triatletas entrenados**

cuyos autores son: **Garnacho MV, Cuenca E, Serra N, Gomis M, Garnacho MA, Pleguezuelos E, Guirao L, Veiga P, García P, Maté JL, Domínguez R.**

ha sido presentada en las VII JORNADAS NACIONALES DE MEDICINA DEL DEPORTE, DE LA PREVENCIÓN AL TRATAMIENTO, celebradas en Zaragoza, los días 24 y 25 de noviembre de 2017.

Lo que certifico a los efectos oportunos en Zaragoza a 25 de noviembre de 2017.

Dr. Pedro Manonelles
Presidente Comité Organizador
VII Jornadas Nacionales SEMED-FEMEDE

Informe del director.

El Dr. Manuel Vicente Garnacho Castaño declara, como director de la tesis doctoral presentada por el doctorando Eduardo Cuenca García titulada "Efecto de la suplementación aguda con zumo de remolacha en el rendimiento aeróbico y anaeróbico", la autenticidad del factor de impacto de los artículos *Journal Citation Reports* presentados, así como su implicación y dedicación en la elaboración de dichos artículos científicos que conforman esta tesis doctoral.

Eduardo ha participado de forma proactiva en la elaboración de los artículos, como queda reflejada en la relación de autores de todos los artículos, figurando como primer autor en uno de ellos.

Concretamente en el primer artículo, como queda reflejado en el artículo original, el doctorando realizó toda la fase experimental, contribuyó a la preparación de todos los materiales y herramientas de análisis, así como aprobó la versión final del manuscrito. (Author's contribution, pag. 11). En el segundo artículo de revisión, el doctorando seleccionó y analizó el contenido de los artículos incluidos en el manuscrito, preparó las figuras, tablas y redactó el manuscrito, editó y revisó el manuscrito, así como aprobó su versión final (Author's contribution, pag. 9). En el tercer artículo el doctorando realizó toda la fase experimental y contribuyó a la escritura de la versión final del manuscrito (Author's contribution, pag. 11).En el cuarto artículo, además de realizar la fase experimental, analizó los datos y escribió el manuscrito (Author's contribution, pag. 9).

A continuación, se detalla la relación de artículos que conforman esta tesis:

Artículo I:

Título: Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts.

Autores: Domínguez R, Maté-Muñoz JL, **Cuenca E**, García-Fernández P, Mata Ordoñez F, Lozano-Estevan MC, Veiga-Herreros P, Fernandes da Silva S, Garnacho-Castaño MV**.** Revista: Journal of the International Society of Sports Nutrition.

DOI: DOI 10.1186/s12970-017-0204-9. Año: 2018

ISSN: 1550-2783; Factor de Impacto (2018): 3,841, Cuartil: Q1

Artículo II:

Título: Effects of a single dose of beetroot juice on cycling time trial performance at ventilatory thresholds intensity in male triathletes.

Autores: Garnacho-Castaño MV, Palau-Salvà G, **Cuenca-García E**, Muñoz-González A, García-Fernández P, Lozano-Estevan MC, Veiga-Herreros P, Maté-Muñoz JL, Domínguez R.

Revista: Journal of the International Society of Sports Nutrition

DOI: doi.org/10.1186/s12970-018-0255-6; Año: 2018

ISSN: 1550-2783; Factor de Impacto (2018): 3,841, Cuartil: Q1

Artículo III:

Título: Effects of beetroot juice supplementation on a 30-second high intensity effort.

Autores: Domínguez R, Garnacho-Castaño MV, **Cuenca E**, García-Fernández P, Muñoz-González A, de Jesús F, Lozano-Estevan MC, Fernandes da Silva S, Veiga-Herreros P, Maté-Muñoz JL**.**

Revista: Nutrients.

DOI: doi:10.3390/nu9121360, Año: 2017

ISSN: ISSN 2072-6643; Factor de Impacto (2017): 4,196, Cuartil: Q1

Artículo IV:

Título: Effects of beetroot juice supplementation on performance and fatigue in a 30-s all-out sprint exercise: A randomized, double-blind cross-over study.

Autores: **Cuenca E**, Jodra P, Pérez-López A, González-Rodríguez L, Fernandes da Silva S, Veiga-Herreros P, & Domínguez R.

Revista: Nutrients.

DOI: doi:10.3390/nu10091222, Año: 2018

ISSN: ISSN 2072-6643; Factor de Impacto (2017): 4,196, Cuartil: Q1

Sinceramente,

Dr. Manuel V. Garnacho Castaño

En Barcelona, a 2 de septiembre de 2019

ARTÍCULOS CIENTIFICOS ORIGINALES

Domínguez et al. Journal of the International Society of Sports Nutrition
(2018) 15:2
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Journal of the International Society of Sports Nutrition

REVIEW Open Access

Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts

Raúl Domínguez[1*], José Luis Maté-Muñoz[1], Eduardo Cuenca[2], Pablo García-Fernández[1], Fernando Mata-Ordoñez[3], María Carmen Lozano-Estevan[1], Pablo Veiga-Herreros[1], Sandro Fernandes da Silva[4] and Manuel Vicente Garnacho-Castaño[2]

Abstract: Beetroot juice contains high levels of inorganic nitrate (NO_3^-) and its intake has proved effective at increasing blood nitric oxide (NO) concentrations. Given the effects of NO in promoting vasodilation and blood flow with beneficial impacts on muscle contraction, several studies have detected an ergogenic effect of beetroot juice supplementation on exercise efforts with high oxidative energy metabolism demands. However, only a scarce yet growing number of investigations have sought to assess the effects of this supplement on performance at high-intensity exercise. Here we review the few studies that have addressed this issue. The databases Dialnet, Elsevier, Medline, Pubmed and Web of Science were searched for articles in English, Portuguese and Spanish published from 2010 to March 31 to 2017 using the keywords: beet or beetroot or nitrate or nitrite and supplement or supplementation or nutrition or "sport nutrition" and exercise or sport or "physical activity" or effort or athlete. Nine articles fulfilling the inclusion criteria were identified. Results indicate that beetroot juice given as a single dose or over a few days may improve performance at intermittent, high-intensity efforts with short rest periods. The improvements observed were attributed to faster phosphocreatine resynthesis which could delay its depletion during repetitive exercise efforts. In addition, beetroot juice supplementation could improve muscle power output via a mechanism involving a faster muscle shortening velocity. The findings of some studies also suggested improved indicators of muscular fatigue, though the mechanism involved in this effect remains unclear.

Keywords: Beet, Ergogenic aids, Exercise, Sport supplement

Background

Because of the increase in competitive equality in high level sport, a 0.6% performance improvement is today considered sufficient to make a difference [1]. In this setting of high competition, athletes often look to nutritional supplements to boost their performance [2]. However, most statements about the potential effects on sport performance or health that appear on the labels of many products are not backed by clear scientific evidence [2]. Because of this, institutions such as the Australian Institute of Sport (AIS) have created a system to classify supplements according to their effects on performance based on confirmed scientific evidence [3]. Thus, dietary supplements assigned to class A have been proven with a high level of evidence to improve exercise performance in certain modalities when taken in appropriate amounts. The only substances in this class are β-alanine, sodium bicarbonate, caffeine, creatine and beetroot juice [4]. However, it is thought that the effect of a given supplement on performance besides the recommended dose may be specific to each sport's modality [5]. This, in turn, will depend on the energy and/or mechanical requirements of each form of exercise such that some supplements will have an ergogenic effect on some types of exercise efforts and have no effects on other types.

The relationship between exercise intensity and time to exhaustion is hyperbolic [6] as it is directly linked to the prevailing energy producing systems during exercise [7]. Thus, depending on their bioenergetics, the different exercise efforts can be classified according to exercise duration. This means we can differentiate between explosive efforts,

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high-intensity efforts and endurance-intensive efforts [8]. Explosive efforts are those lasting under 6 s in which the main energy metabolism pathway is the high-energy phosphagen system and there is some participation also of glycolysis [9, 10], which gradually contributes more energy until 50% at 6 s [9]. High-intensity efforts are those of duration longer than 6 s and shorter than 1 min [11]. These efforts are characterized by a major contribution of glycolytic metabolism and smaller contribution of high-energy phosphagens and oxidative phosphorylation [8]. Finally, intensive endurance efforts are those lasting longer than 60 s and whose main energy producing system is oxidative phosphorylation [8].

Beetroot juice is used as a supplement because it may serve as a precursor of nitric oxide (NO) [12]. The mechanism of NO synthesis is thought to be via the catabolism of arginine by the enzyme NO synthase [13]. Effectively, arginine supplementation has been shown to increase NO levels [14]. An alternative mechanism of NO genesis is mediated by inorganic nitrate (NO_3^-). This means that the high amounts of NO_3^- present in beetroot juice are able to increase NO levels in the organism.

In the mouth, some 25% of dietary NO_3^- is reduced by NO_3^- reductase produced by microorganisms [15] to nitrite (NO_2^-) [16]. This NO_2^- is then partially reduced to NO through the actions of stomach acids which is later absorbed in the gut [17]. Some of this NO_2^- enters the bloodstream, and, in conditions of low oxygen levels, will be converted into NO [18] (Fig. 1).

Nitrous oxide has numerous physiological functions including haemodynamic and metabolic actions [19, 20]. Mediated by guanylyl cyclase [21], NO has an effect on smooth muscle fibres causing blood vessel dilation [22]. This vasodilation effect increases blood flow to muscle fibres [23] promoting gas exchange [24]. NO also induces gene expression [25], enhancing biogenesis [26] and mitochondrial efficiency [27]. All these effects can favour an oxidative energy metabolism. In effect, though not all [28–31], numerous investigations have noted that beetroot juice supplementation boosts performance in exercise modalities involving intensive endurance efforts in which the dominant type of energy metabolism is oxidative [24, 27, 32–45].

To date, several reviews of the literature have assessed the effects of beetroot juice supplements on physical exercise [12, 46–49]. In addition, given that NO can potentiate the factors that limit performance when executing actions in which the predominant metabolism is oxidative, two recent reviews have explored the positive effects of this form of supplementation on endurance exercise [50, 51]. Thus, the different studies showed that beetroot juice supplementation was effective at: lowering VO_2 by −6% during a swimming test conducted at an intensity equivalent to the

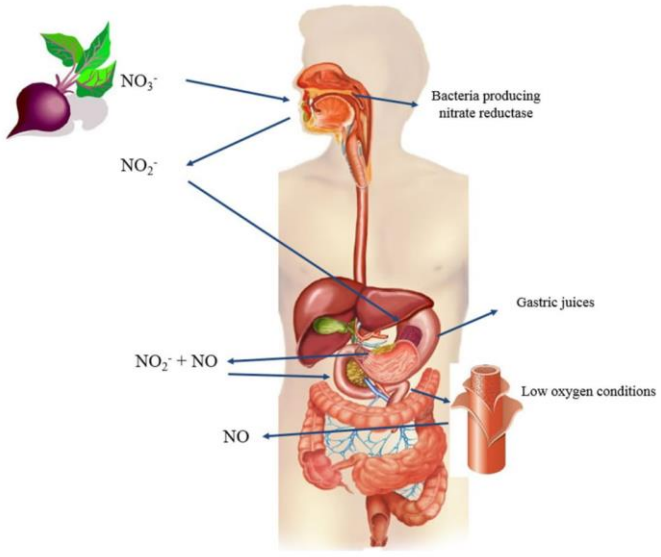

Fig. 1 Conversion of NO_3^- in beetroot juice to NO. The diagram shows how ingested NO_3^- is transformed by bacteria in the mouth containing nitrite reductase to NO_2^-. Once in the gut, NO_2^- enters the bloodstream and, under conditions of hypoxia, is used to generate NO

ventilatory threshold (VT) [27]; lowering VO_2 by −3% during a kayaking test conducted at 60% VO_{2max} [38] and during a cycle ergometry test conducted by recreation sport athletes [45] and cyclists [34] at 45–70% VO_{2max}; increasing performance by 12–17% in cycle ergometry tests until exhaustion conducted at intensities of 60 to 90% VO_{2max} by recreation sport athletes [37, 42], and by 22% when conducted at a 70% intensity between VT and VO_{2max} [36]; and finally, improving times by 2.8% in trained cyclists conducting cycle ergometery tests of 4 km [33], 10 km (1.2%) [34], 16 km (2.7%) [33] and 50 miles (0.8%) [35]. However, besides the effects of NO mentioned above, other impacts need to be considered. Accordingly, it has been described that the effect of increased blood flow induced by NO is specific to type II muscle fibres [20]. Moreover, in type II muscle fibres, beetroot juice intake has been found to improve the release and later reuptake of calcium from the sarcoplasmic reticulum [52]. This could translate to an increased capacity for muscle strength production of these type II muscle fibres. Such effects of NO could mean a physiological advantage for efforts involving the recruitment of type II muscle fibres, such as intermittent, high-intensity efforts. Hence, given the scarce yet growing number of studies that have addressed the effects of beetroot juice supplementation on this type of intermittent, high-intensity effort [38, 53–60], here we review the results of experimental studies that have specifically examined in adults (whether athletes or not) the effects of beetroot juice supplementation on intermittent, high-intensity efforts.

Methodology

We identified all studies that have assessed the effects of BJ supplementation on intermittent, high-intensity efforts by searching the databases Dialnet, Elsevier, Medline, Pubmed and Web of Science published up until March 31, 2017 using the keywords: beet OR beetroot OR nitrate OR nitrite (concept 1) AND supplement OR supplementation OR nutrition OR "sport nutrition" (concept 2) AND exercise OR sport OR "physical activity" OR effort OR athlete (concept 3).

Two of the present authors (E.C and P.G-F) first eliminated duplicate articles and then removed descriptions of studies that were not experimental, were not written in English or Spanish, or were published before 2010. This meant that all the studies reviewed were published over the period January 1, 2010 to March 31, 2017. Next, these two same authors applied a set of exclusion criteria to ensure the selection only of studies specifically designed to assess the effects of BJ supplementation on intermittent, high-intensity efforts:

- Studies performed in non-adults (samples including subjects aged <18 or >65 years).
- Studies conducted in vitro or in animals.
- Studies in which the direct effects of BJ were not determined.
- Studies in which impacts were examined on exercises that did not comply with the characteristics of intermittent, high-intensity efforts.

If there was disagreement about whether a given study met the inclusion/exclusion criteria, the opinion of a third researcher (F.M-O) was sought.

Results

Study selection

Of 738 studies identified in the search, 359 were left after eliminating repeated records. Once, the titles and abstract of these 359 publications were reviewed, 212 full text articles were indentified and retrieved for assessment, of which 9 articles met the elegibility criteria (Fig. 2).

Study characteristics

The nine studies selected for our review included a total of 120 subjects, 107 of whom were men and 13 women.

In five of these studies [38, 53, 54, 57, 59], the effects of a single beetroot juice supplement (acute effects) were assessed. The supplement was taken 120 min before exercise in one study [53], 150 min before exercise in two [57, 59] and 180 min before exercise in the remaining two [38, 54].

In the remaining four studies, the effects of chronic beetroot juice supplementation were examined [55, 56, 58, 60]. The supplementation periods were 5 days in one study [60], 6 days in two [55, 58] and 7 days in the fourth study [56].

Doses of NO_3^- ingested ranged from ~5 mmol [38] to ~11.4 mmol [57]. In addition, one study examined the efficacy of beetroot juice taken separately or in combination with sodium phosphate [55].

In four of the nine studies reviewed, participants were competition athletes [38, 55, 57, 59] and in the other five they were recreation sport or low-level competition athletes [53, 54, 56, 58, 60]. Only one of the study populations included athletes of individual sports modalities [38], the rest of the studies were conducted in players of team sports [53–60].

The tests used to assess performance were a 30-s duration cycle ergometer test in one [59] and high-intensity, intermittent exercises in the remaining studies with work intervals ranging from 6 s [58] to 60 s [60] and rest periods from 14 s [56] to 4 min [60]. The types of tests employed were running at maximum speed in three studies [55–57], cycle ergometry in four [53, 54, 59, 60], one of which was an isokinetic test [59], a kayak ergometer test in one [38] and bench press strength training in the remaining study [58].

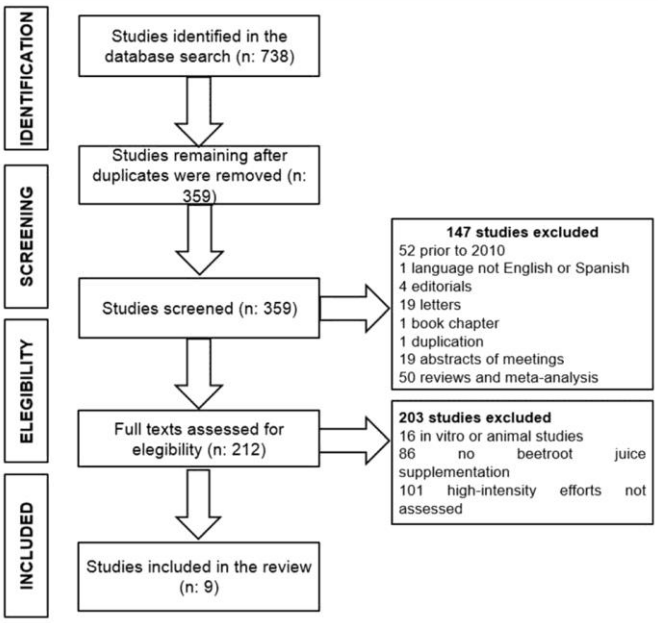

Fig. 2 Article selection

The beetroot juice intervention led to significantly improved performance in four of the studies [54, 56, 58, 60], while in another four no such effects were observed [38, 55, 57, 59]. In the remaining study, an ergolytic, or reduced performance, effect was noted in relation to the placebo treatment.

Study results

In Table 1 we summarize the results of the nine studies reviewed and provide details on the participants, experimental conditions, supplement regimens, and performance tests employed.

Discussion

Effects of chronic supplementation with beetroot juice on intermittent, high-intensity exercise efforts

Four of the studies reviewed tested the effects of taking beetroot juice supplements for 5 to 7 days on intermittent, high-intensity efforts [55, 56, 60] or on a resistance training session [58]. Three of these studies detected a significant effect of beetroot juice supplementation [56, 58, 60] while in the remaining study, no significant difference compared with the placebo was noted [55].

Effects of chronic supplementation with beetroot juice on resistance training

Resistance training is used to improve muscular hypertrophy, strength, power and muscular endurance [61]. Training sessions targeting muscle hypertrophy include workloads of around 70–85% 1 RM and 8–12 repetitions, while those aiming to improve muscular endurance include loads of around 50% 1 RM and some 15–25 repetitions [62]. Such exercise sessions are largely dependent on glycolytic metabolism; the lactate threshold in resistance training exercises such as half squat is detected at ~25% 1 RM [63, 64]. To determine the effects of 6 days of beetroot juice supplementation (6.4 mmol NO_3) on resistance training sessions designed to improve local muscular hypertrophy and endurance, in the study by Mosher et al. reviewed here [58], the number of bench press repetitions accomplished in three sets using loads equivalent to 60% 1 RM was recorded. Results indicated that supplementation increased the number of repetitions in the three exercise sets improving session performance by 18.9%.

In an earlier investigation, the effects of sodium bicarbonate supplements were assessed in a similar study to the one by Mosher et al. [58]. Subjects performed 3 sets until exhaustion with loads of 10–12 RM in three exercises

Table 1 Summary of the results obtained in studies examining the impacts of beetroot juice supplements on intermittent high intensity exercise performance

Reference	Subjects	Study design	Dose	Exercise test	Results
Muggeridge et al. [38]	Trained kayakers (male, $n = 8$) (VO_{2peak} 49.0 ± 6.1 ml·kg·min^{-1})	Single-blind, randomized, cross-over	5 mmol NO_3^- (180 min before)	Kayak ergometer: 5 × 10 s sprint-rest 50 s	+4% average power (420 ± 23 vs 404 ± 24 W)
Martin et al. [53]	Recreation team sport players (male, $n = 16$) (VO_{2peak} 47.2 ± 8.5 ml·kg·min^{-1})	Double-blind, randomized, cross-over	6.4 mmol NO_3^- (120 min before)	Cycle ergometer: sets until exhaustion of 8 s– rest 30 s	−13% reps (13 ± 5 vs 15 ± 6) and −17% total work (49.2 ± 24.2 vs 57.8 ± 34.0 kJ)
Aucouturier et al. [54]	Recreation team sport players (male, $n = 12$) (VO_{2peak} 46.6 ± 3.4 ml·kg·min^{-1})	Single-blind, randomized, cross-over	10.9 mmol NO_3^- (180 min before)	Cycle ergometer: sets until exhaustion of 15 s at 170% MAP–rest 30 s	+20% reps* (26.1 ± 10.7 vs 21.8 ± 8.0) and 18% total workload* (168.2 ± 60.2 vs 142.0 ± 46.8 kJ)
Buck et al. [55]	Amateur team sport players (female, $n = 13$) (VO_{2peak} not specified)	Double-blind, randomized, Latin-square	BJ: 6.4 mmol NO_3^- (6 days) BJ + SP: 6.4 mmol NO_3^- + 50 mg·kg lean mass SP (6 days)	PRE, MID and POST simulation team sport matches: 6×(20 m sprint + rest 25 s)	BJ: −0.2% total sprint time per set (69.8 ± 4.9 vs 69.97 ± 4.2) BJ + SP: −2% total sprint time per set (68.9 ± 5.1 vs 69.97 ± 4.2)
Thompson et al. [56]	Recreation team sport players (male, $n = 16$) (VO_{2peak} 50 ± 7 ml·kg·min^{-1})	Double-blind, randomized, cross-over	12.8 mmol NO_3^- (7 days)	MID and POST simulated team-sport matches: 2×[5×(6 s cycle ergometry sprint + rest 14 s)]	5% work volume at MID* (63 ± 20 vs 60 ± 18 kJ), 2% POST (60 ± 17 vs 59 ± 16 kJ) and 4% whole session* (123 ± 19 vs 119 ± 17 kJ)
Clifford et al. [57]	Competition team sport players (male, $n = 20$) (VO_{2peak} not specified)	Double-blind, independent groups design	11.4 mmol NO_3^- (150 min before)	2xRST: 20×(30 m sprint–rest 30 s)	-1% average sprint time RST1 (4.65 ± 0.3 vs 4.7 ± 0.2 s) and −2% RST2 (4.66 ± 0.2 vs 4.77 ± 0.2 s) and −2% fastest sprint RST1 (4.41 ± 0.2 vs 4.48 ± 0.1 s) and −3%RST2 (4.38 ± 0.2 vs 4.53 ± 0.2 s)
Mosher et al. [58]	Recreation sport players (male, $n = 12$) (VO_{2peak} not specified)	Double-blind, randomized, cross-over	6.4 mmol NO_3^- (6 days)	Bench press: 3× (maximum number reps at 60% 1 RM)	+ 19% weight lifted in session and improved no. of reps S1*; S2*; S3* and whole session. * improvements not specified
Rimer et al. [59]	Competition sport players (male, $n = 13$) (VO_{2peak} not specified)	Double-blind, randomized, cross-over	11.2 mmol NO_3^- (150 min before)	Isokinetic cycle ergometer: Wingate 30-s test	-1% peak power (1173 ± 255 vs 1185 ± 249 W) and −1% total work (22.8 ± 4.8 vs 23 ± 4.8 W)
Wylie et al. [60]	Recreation team sport players (male, $n = 10$) (VO_{2peak} 58 ± 8 ml·kg·min^{-1})	Double-blind, randomized, cross-over design	8.4 mmol NO_3^- (5 days)	Cycle ergometer: 24 x (6 s sprint–rest 24 s) Cycle ergometer: 7 x (30 s sprint–rest 4 min) Cycle ergometer: 6 x (60 s sprint–rest 60 s)	+5% mean average power* (568 ± 136 vs 539 ± 136 W) and +1% mean peak power (792 ± 159 vs 782 ± 154 W) in 24 x (6 s sprint–rest 24 s); −1% mean average power (558 ± 95 vs 562 ± 94 W) and −1% mean peak power (768 ± 157 vs 776 ± 142 W) in 7 x (30 s sprint–rest 4 min)

BJ Beetroot juice, *MID* Half-time simulation match, *n* Sample size; *no* Number, NO_3^- nitrate concentration in the drink, *MAP* Maximum aerobic power, *POST* End simulation match, *PRE* Before simulation match, *Rep* Repetition, *RST* Repeated sprint test, *SP* Sodium phosphate, VO_{2peak} Peak oxygen consumption, * statistically significant differences

targeting the lower limbs [65]. Results indicated that, like the beetroot juice, sodium bicarbonate supplementation led to more repetitions in the session [65]. However, in parallel with the increasing number of repetitions, blood lactate concentrations also rose (~2.5 mmol) [65]. This was not observed in Mosher's study [58].

If we consider the nature of resistance training, the athlete passes from a resting condition to a situation demanding high energy levels during the first repetitions of a set. Because the phosphagen system is the main energy pathway in rest-exercise transitions [66], phosphocreatine reserves may be depleted in response to a resistance training

exercise set. Recovering these reserves takes some 3–5 min [67]. Given that phosphocreatine resynthesis is dependent on oxidative metabolism [68] and that beetroot juice has an ergogenic effect on exercise modalities with a major oxidative metabolism component [50], it could be that this supplement accelerated this recovery during the rest period in Mosher's study (2 min) and thus avoided progressive phosphocreatine depletion throughout the session. In turn, this faster rate of resynthesis would attenuate the increasing levels of adenosine diphosphate (ADP) and inorganic phosphates [68]. Both these metabolites have been associated with the appearance of muscular fatigue [69]. Hence, by delaying the build-up of critical levels of these metabolites, the appearance of fatigue will be delayed and this will allow for more repetitions in sets until exhaustion [58]. NO_3^- supplementation could also improve muscle efficiency and contractile capacity by promoting the release of calcium from the sarcoplasmic reticulum in the muscle cells and its reuptake [52, 69]. Thus, a train of action potentials leading to an increased supply of calcium to the muscle fibre will increase the strength of muscle contraction [13].

Effects of chronic supplementation with beetroot juice on intermittent high-intensity exercise efforts

Some sport modalities such as team, racket or combat sports require bursts of high-intensity efforts followed by rest periods. Thus, in team sports, high-intensity efforts (~3–4 s) are interspersed with variable active rest periods [70]. In racket sports like tennis, efforts last 7–10 s and rest periods 10–16 s (between points) and/or 60–90 s (side changes) [71]. Finally, in combat sports more intense efforts are 15–30 s long and active rest periods are 5–10 s long every 5 min [72]. In all these sports modalities, the capacity to repeat high-intensity efforts with only short recovery periods is considered a performance indicator [73]. This means that higher level athletes are able to maintain performance in successive high-intensity intervals over a long time period [74].

To find out if beetroot juice supplementation would improve this ability to repeat high-intensity efforts during a team sport match, Thompson et al. [56] administered beetroot juice over 7 days to a group of athletes (12.8 mmol NO_3^-). The performance test consisted of two blocks of five 6-s sets of sprints on a cycle ergometer with 14-s active recovery periods in the middle and end of a simulated match lasting 2 × 40 min [56]. The results of this study indicated a total work volume improved by 3.5% in the whole session, though this improvement was greater at the end of the first half (at half time).

If we again consider the nature of this type of exercise, it has been established that it involves the recruitment of type II muscle fibres [75, 76], which are more powerful though show more fatigue than type I units [77]. This lesser resistance to fatigue has been related to reduced blood flow and myoglobin concentrations in these muscle fibres compared to type I. Hence, type II muscle fibres are designed to promote non oxidative pathways and have shown a greater creatine storage capacity [78] for an enhanced metabolism of phosphocreatine [79] and proteins with a buffering effect at the intracellular level such as carnosine [80], favouring a glycolytic type metabolism.

Animal studies have shown that increased blood flow in response to NO_3^- supplementation is greater in type II compared to type I muscle fibres [20]. This greater irrigation and oxygen availability in the recovery period along with a greater creatine storage capacity of motor type II units [78] (promoting phosphocreatine resynthesis [79]) means that during an exercise effort followed by a short rest period (14 s), beetroot juice supplementation could delay phosphocreatine depletion during successive sprints and explain the improvements noted by Thompson et al. [56].

Despite such greater effects of NO_3^- supplementation on type II versus type I muscle fibres, animal studies have also shown that effects on calcium release and reuptake in the muscle cell sarcoplasmic reticulum is greater in type II than type I muscle fibres [52]. Accordingly, because of the important role of type II muscle fibres during sprints [75, 76], supplementation could have led to an improved capacity to generate muscle power and thus explain the significant improvements in performance observed by Thompson's group.

Buck et al. [55] examined the effects of 6 days of supplementation with beetroot juice (6.4 mmol NO_3^-) or sodium phosphate (50 mg·kg lean mass) on performance in a test consisting of repeated sprints as 6 sets of 20 m and 25-s of rest between sets in the middle and end of a simulated match lasting 60 min. The beetroot juice intervention did not improve performance at these sprints, yet did do so when taken along with sodium phosphate (2%) compared with placebo, though this improvement was of lesser magnitude than when the subjects only took sodium phosphate supplements (5%). These findings suggest that, unlike beetroot juice, sodium phosphate intake may have an ergogenic effect in this protocol. If we compare the tests used by Buck et al. [55] and Thompson et al. [56], work periods were shorter (2–3 vs 6 s), while rest periods were longer (25 vs 14 s). Therefore it could be that 2–3 s efforts lead to a significantly lower reduction of phosphocreatine reserves at the end of these efforts. Further, the 25 s of rest approaching the 30 s in which the recovery of 50% of phosphocreatine stores takes place [67], may have been sufficient to stabilize reserves of phosphocreatine and therefore avoid the appearance of fatigue [81].

Another study investigated the effects of longer term supplementation (5 days) with beetroot juice (8.4 mmol NO_3^-), this time on performance in a repeated high-

intensity test [60]. These authors sought to determine supplementation effects on different exercise protocols. Subjects performed a session consisting of twenty four 6-s sets of work and 24 s of rest between sets, a second session of two 30-s sets of work and 2 min of rest between sets and a third session of six 6-s sets and 60 s of rest between sets. As did Thompson et al. [56], Wylie et al. [60] selected 6-s exercise sets in the first session though rest intervals were longer (24 vs 14 s). Another difference was that the participants had not first undergone fatigue (in the simulated team sport match) before the performance test. Notwithstanding, results were similar in that mean power generated in the sets over a whole session improved by ~7%. However, improvements across the 24 × 6–24 protocol were not comparable to those recorded in the other two tests, in which no significant improvements were recorded.

In the test protocols including 30-s and 60-s work efforts, beetroot juice supplementation resulted in no improvements in any indicators of performance [60]. These protocols consisting of longer duration work intervals mainly involve a glycolytic type metabolism and in smaller measure elicit the high-energy phosphagen system. An increase in glycolysis leads to increased H^+ production, lowering pH [82]. To avoid increasing acidosis, a series of responses targeted at reducing phosphofructokinase take place including diminished glycolysis [83] and phosphocreatine resynthesis [84], and muscle contractibility modifications [85]. Such responses manifest as reduced non aerobic metabolism or a reduced capacity for muscle power and strength, in other words, fatigue [86]. Supplements such as β-alanine (which increases muscle carnosine concentrations [87], a protein that acts as a buffer inside the cell [88]) and sodium bicarbonate [89] (main extracellular buffering agent) have shown ergogenic effects on performance at high-intensity efforts involving the predominance of glycolytic metabolism [90]. The combined effect of these supplements is greater than the impact of each supplement on its own [91].

Although beetroot juice supplementation induces vasodilation and increased blood flow (in type II muscle fibres, recruited mainly in exercise bouts of 30 to 60 s duration), increasing available oxygen in the muscles, rather than being activated because of a lack of oxygen (anaerobiosis), non-oxygen dependent pathways are activated because of a greater demand for energy production via oxidative phosphorylation. Thus, these effects, although they potentiate oxidative phosphorylation, have no repercussions on glycolytic energy metabolism. Hence, as beetroot juice has no alkalizing effect supplementation with this product is unable to reduce acidosis, as the main factor limiting performance at efforts lasting 30–60 s. However, potentiating effects on aerobic metabolism increases the speed of phosphocreatine resynthesis, dependent on oxidative phosphorylation. This means it may be effective for repeated high-intensity efforts whose duration is close to 6–10 s, in which high energy phosphagens contribute mainly to the metabolism [92] and the work volume is sufficient to cause significant depletion, which when faced with short rest intervals leads to progressive depletion and consequently to fatigue. Accordingly, beetroot juice supplements can have an ergogenic effect when exercise efforts are intermittent, maximum intensity, short-duration (6–10 s) and interspersed with brief recovery periods (<30 s).

Effects of acute beetroot juice supplementation on intermittent high-intensity efforts

Five of the studies reviewed here were designed to analyze the effects of a single beetroot juice supplement on intermittent high-intensity exercise efforts [38, 53, 54, 57, 59]. Aucouturier et al. [54] administered the supplement (~10.9 mmol NO_3^-) to a group of recreation athletes 180 min before performing sets until exhaustion consisting of 15 s of pedalling at 170% VO_{2max} followed by 30-s rest periods. The authors reported that the beetroot supplement gave rise to improvements close to 20% in the number of repetitions performed and the total work completed in the session [54]. Besides the number of sets completed and the work accomplished, these authors measured red blood cell concentrations at the microvascular level. The beetroot juice, apart from improving performance, was found to increase microvascularization. Such improvements are considered a beneficial effect on oxygen exchange in the muscle [93]. Accordingly, these oxygen availability improvements produced at the muscular level could have potentiated oxidative phosphorylation during rest periods, and, given their brief duration, could have increased phosphocreatine resynthesis when subjects took the supplement rather than the placebo. Thus, supplementation would have delayed the depletion of phosphocreatine reserves and this effect was likely the cause of the improvements observed in the repeated sets of intermittent sprints [94, 95].

As did Aucouturier et al. [54], Muggeridge et al. [38] examined the effect of beetroot juice (5 mmol NO_3^-) taken 180 min before an intermittent effort consisting of 5 sets of 10 s in a kayak ergometer with 50-s interset rest periods. In this study, though supplementation seemed to have a greater effect on the power generated in the last two sets, the improvement noted lacked significance. However, if we compare this study with the study by Aucouturier et al. [54], work periods in the Muggeridge study [38] were shorter (10 vs 15 s) and rest periods were much longer (50 vs 30 s). Ten second maximum intensity intervals have a significantly reduced capacity compared with 15s intervals to deplete phosphocreatine reserves. Moreover, the rate of phosphocreatine replacement has a first phase in which up to 50% of these reserves can be replenished in 30 s and

100% in 3–5 min [67]. Also if we consider that the main effect of beetroot juice supplements is linked to an improved rate of phosphocreatine resynthesis, it is possible that as there is less depletion and a rest period in which there is almost complete recovery of phosphocreatine reserves, supplementation could not have exerted any beneficial effect in the study by Muggeridge et al. [38]. However, despite the short work periods and relatively long recovery periods and the fact that the power developed in the last sets showed an improved trend following supplementation, it is possible that lengthening intervals in a set until exhaustion would have been beneficial and given rise to similar results to those observed by Aucouturier et al. [54].

Rimer et al. [59] assessed the effects of acute supplementation (150 min before exercise) with beetroot juice (11.2 mmol NO_3^-) on performance in a maximal intensity 3-s test on an isoinertial cycle ergometer and a 30-s test on an isokinetic cycle ergometer. Supplementation was effective at improving pedalling cadence, and thus the power generated, in the 3-s test. However, no such effect was observed in the isokinetic test.

The improvements noted by Rimer's group in the 3-s test affected pedalling cadence. Because of the link between such improvements and an increase in muscle shortening velocity [96] and the proposal that NO could increase this velocity [97, 98], the authors suggested that beetroot juice could have a beneficial effect on power output [59]. This rationale was also used to explain the lack of changes produced in the 30-s test in which pedalling cadence was fixed at 120 rpm. This means that any improved power production in the isokinetic test could only occur if there was an increase in power at a constant shortening velocity [59], since power equals force times velocity.

In a later investigation performed in CrossFit athletes, it was reported that supplementation with NO_3^- salts (8 mmol NO_3) rather than beetroot juice was able to improve performance in a 30-s cycle ergometry test [99]. However, unlike the 30-s test used by Rimer et al. [59], the test was isoinertial. The difference between the 2 cycle ergometers is that while in the isokinetic test pedalling cadence is prefixed and improvements only in strength are possible, in an isoinertial test the workload is fixed and any power improvements produced manifest as improvements in pedalling cadence. Given that beetroot juice supplementation could improve power development as a consequence of a reduced muscle shortening velocity [59, 97, 98], the isokinetic cycle ergometer is perhaps not sufficiently sensitive to assess the effects of this supplementation. Considering the beneficial effects on cadence and power output observed in the cycle ergometry 3-s [59] and 30-s [99] tests, it seems that beetroot juice supplementation could have a beneficial effect on this type of effort.

In a fourth study, Clifford et al. [57] assessed the effects of a single intake of beetroot juice on performance in a test of 20 sets of 30 m sprints interspersed with 30-s rest periods. These authors observed no ergogenic effects of the supplementation. However, if we look at the characteristics of the test employed by the researchers, we find that the work periods (close to 3 s) together with the 30 s recovery periods could be sufficient for the subjects to have recovered their phosphocreatine levels in the rest intervals, minimizing the possible ergogenic effects of the supplementation.

A novel indicator used in this study by Clifford et al. [57] was the counter-movement jump (CMJ) test performed before the intermittent velocity test and in the rest periods. Performance in this test is determined by the contractile properties of muscle and by neuromuscular control of the entire musculoskeletal system [100]. Given that fatigue reflects the incapacity of the neuromuscular system to maintain the level of power required [101], losses in CMJ height at the end of exercise are taken as an indicator of muscular fatigue [102].

In the study by Clifford's group [57], it was observed that the protocol of intermittent sprints gave rise to muscular fatigue. This fatigue can be the outcome of deficiencies in the muscle's contractile mechanism [101, 103]. Alternatively, strong eccentric actions of the hamstring muscles during sprints may produce muscle damage [104] and therefore modify the structure of the muscle fibre's sarcomeres. Thus, any loss in CMJ height could indicate muscle damage. While CMJ was monitored after the protocol of 20 sets of 30 m with 30-s rest periods, a greater recovery of CMJ height was observed in the supplementation group. This suggests that beetroot juice could help preserve muscle structure during high-intensity efforts. Another explanation could be related to the vasodilation effect of beetroot juice [50] possibly helping muscle regeneration during early recovery. In future work, biomarkers of muscle damage or inflammation need to be examined.

In the fifth study, Martin et al. investigated the effects of beetroot juice (6.4 mmol NO_3^-) on repetitive sets until exhaustion each consisting of 8 s of work followed by 30 s of rest on a cycle ergometer [53]. No effects were detected on power output in the different sets. Moreover, a lower number of sets was accomplished in the session for the supplementation group versus placebo group. In effect, this was the only study to describe an ergolytic effect of beetroot juice. The authors argued that because of the scarce contribution of oxidative phosphorylation to energy metabolism during high-intensity efforts and that the ergogenic potential of this supplement is related to potentiating oxidative pathways, no beneficial effects are produced on this type of physical action.

The results of the investigation by Martin et al. [53] conflict with those of others who did observe beneficial effects on performance in similar tests [54, 56, 58, 60]. Beetroot juice was taken 120 min before exercise. This regimen is not appropriate, as peak NO_2^- levels are produced 2–3 h after ingestion and it is recommended that supplementation should be taken at least 150 min–180 min before the high-intensity effort [32, 50]. Effectively, Aucouturier et al. [54] used a test of similar characteristics but the beetroot supplement was taken 180 min before the exercises, as recommended.

Conclusions

To date, few studies have examined the effects of supplementation with beetroot juice on short-duration high-intensity exercise efforts [38, 53–60] and observations so far will need confirmation in future studies:

- Supplementation with beetroot juice has been shown to diminish the muscular fatigue associated with high-intensity exercise efforts, though it is not known if this is achieved by reducing fatigue and muscle damage and/or promoting muscle regeneration postexercise.
- When faced with exercise efforts that could considerably deplete phosphocreatine reserves (sets of resistance training or repetitive sprints of around 15 s interspersed with short rest periods) and given that phosphocreatine resynthesis requires an oxidative metabolism, beetroot juice could help the recovery of phosphocreatine reserves and thus avoid its depletion during repeated efforts. In parallel, supplementation would limit the build-up of metabolites such as ADP and inorganic phosphates, which are known to induce muscular fatigue.
- Beetroot juice has been shown to improve the release and reuptake of calcium at the sarcoplasmic reticulum. This could help the power production associated with improvements in muscle shortening velocity. Non-isokinetic ergometers (in which movement velocity is not assessed) are sensitive to such improvements in power generation.

Study limitations

The main limitation of our review is the scarcity of studies that have examined the effects of beetroot juice supplementation on intermittent, high- intensity exercise. This limitation is also magnified by the varied design of the few studies available including different supplementation doses and regimens.

Future lines of research

- As it has been proposed that beetroot juice supplementation improves phosphocreatine resynthesis during the brief rest periods included in protocols of intermittent high-intensity exercise, future studies are needed to confirm via a muscle biopsy phosphocreatine levels during repeated high-intensity efforts.
- To examine the possible beneficial effect of beetroot juice on muscle shortening velocity reflected as improved pedalling cadence, future studies need to assess the ergogenic effect of this supplement in a single, constant-load test on an inertial cycle ergometer.
- To elucidate the mechanism whereby beetroot juice diminishes muscular fatigue and improves recovery from this fatigue, the effects of ingesting NO_3^- on biomarkers of inflammation and muscle damage need to be addressed.
- According to the results of the study in which an ergolytic effect was produced in response to a single dose of beetroot juice administered 120 min before exercise, future investigations should determine the most appropriate timing of supplementation to optimize its ergogenic potential.
- Finally, owing to the possible beneficial impacts of beetroot juice, we will need to assess the interactions of beetroot juice with other supplements of proven ergogenic effects in this type of exercise effort such as caffeine, creatine, β-alanine and sodium bicarbonate.

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RESEARCH ARTICLE Open Access

Effects of a single dose of beetroot juice on cycling time trial performance at ventilatory thresholds intensity in male triathletes

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Abstract

Background: Beetroot juice (BJ) is classified as a high-level supplement for improving sports performance. There is some controversy over the benefits of BJ supplementation for endurance exercise performance, especially when referring to well-trained athletes. This study examines the effects of acute BJ supplementation on cardioventilatory responses, exercise economy/efficiency, slow component of oxygen uptake, time trial performance, blood lactate, energy consumption, and carbohydrate and fat oxidation.

Methods: Twelve well-trained, male triathletes (aged 21–47 yr) were assigned in a randomized, double-blind, crossover design to receive 70 ml of BJ (6.5 mmol NO_3^-) or placebo (PL). Three hours after taking the supplement, participants completed an endurance test on a cycle ergometer at a constant work rate (W) corresponding to first ventilatory threshold (VT1) (30 min) and second ventilatory threshold (VT2) time trial (~ 15 min).

Results: Maximal oxygen uptake was 54.78 ± 3.13 $mL \cdot min^{-1} \cdot kg^{-1}$, and gross efficiency was > 22% at each load intensity and experimental condition. No significant interaction effect (supplement*intensity) was observed on any of the cardioventilatory variables, efficiency/economy, VT2 time trial, energy expenditure, carbohydrate oxidation and fat oxidation ($p > 0.05$).

Conclusion: Our findings do not support an improvement in the variables examined in response to acute BJ supplementation. Probably, higher doses are needed for improving time trial performance in male triathletes during a cycle ergometer test conducted at a load intensity equivalent to the first and second ventilatory threshold.

Keywords: Cardioventilatory responses, Gross mechanical efficiency, Cycling efficiency, Slow component, Energy expenditure

Background

Beetroot juice (BJ) is classified as a supplement of high scientific evidence for improving sports performance [1]. It is characterized by its high nitrate content (NO_3^-) which, after ingestion, is actively extracted and concentrated in the saliva. Here NO_3^- is reduced to nitrite (NO_2^-) by bacteria in the mouth. In turn, NO_2^- may be further reduced in the stomach and muscle to nitric oxide (NO) [2, 3]. NO is an important signaling molecule with a key role in several physiological processes which may affect exercise performance such as regulating tissue blood flow, muscle contraction, respiration and mitochondrial biogenesis, and muscle glucose uptake [4].

In animal studies, it has been demonstrated that NO_3^- supplementation elevates skeletal muscle O_2 delivery and improves vascular control during exercise predominantly in fast-twitch type II muscles. Furthermore, NO_3^- supplementation improves metabolic control [5]. A human study has suggested that NO_3^- supplementation may enhance physiological and functional responses in type II muscle fibers [6]. These potential physiological mechanisms induced by

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NO_3^- supplementation on type II muscle fibers could justify, at least in part, improvements in performance during intense exercise in healthy adults, and for improving functional capacity in senescent and patient populations [6].

It has been well established that performance at endurance exercise is linked to maximum oxygen uptake (VO_{2max}), lactate threshold, ventilatory threshold (VT), exercise economy/efficiency [7–11] and VO_2 kinetics [12]. Acute and chronic supplementation with NO_3^- has been shown to reduce O_2 cost in various forms of exercise, with different supplementation protocols and at different exercise intensities. NO_3^- reduced O_2 cost during knee-extensor exercise (6 days, 0.5 l/day NO_3^-, 5.1 mmol/day) [13], decreased during high intensity exercise(6 days, 0.5 l/day NO_3^-, 5.5 mmol/day) [14] and submaximal exercise (1 day and 15 days, 0.5 l/day, 5.2 mmol/day of NO_3^-) [15] in cycle ergometer, and diminished during walking and moderate- and severe-intensity running (6 days, 0.5 l/day NO_3^-, ~ 6.2 mmol/day) [16]. In addition, NO_3^- supplementation improved muscle contractile efficiency, increased time to exhaustion by 25%, reduced the amplitude of the VO_2 slow component by 50% [13], and diminished cycle time trial in trained (6 days, 140 ml/day NO_3^-, ~ 8 mmol/day) [17] and competitive cyclists (1 day, 0.5 l NO_3^-, ~ 6.2 mmol) [18]. Because of these findings, it has been proposed that BJ supplementation could have an ergogenic effect in athletes [1] especially when executing long-duration, endurance exercise modalities [19].

There is some controversy over the benefits of BJ supplementation for endurance exercise performance, mainly when referring to highly-trained athletes. In a study performed in elite cyclists, it was found that BJ (6 days, 0.5 l/day NO_3^-, ~ 0.5 g/day, 820 KJ per drink) failed to improve performance, exercise economy and VO_2 kinetics measured in a 2 h preload test and a 400 kcal time trial [20]. Cermak et al. (2012) [21] also observed that acute BJ intake (500 ml, ~ 6.2 mmol NO_3^-) did not improve power, time-trial performance or heart rate response in a 1 h cycle time trial in trained cyclists.

International Olympic Committee (IOC) consensus statement [22] determines that improvements in performance after acute BJ supplementation are commonly detected within 2–3 h following a NO_3^- bolus of 5–9 mmol (310–560 mg) [23]. Longer periods (> 3 days) of NO_3^- supplementation appears to increase sport performance [24, 25], especially, when performance gains appear harder to achieve in highly-trained athletes [12]. Higher doses of NO_3^- (> 8 mmol) have shown to improve performance in trained rowers [23]. There are some uncertainties for the dose-response relationship exists between biological mechanisms and acute BJ supplementation for improving endurance performance in well-trained athletes. The differences observed between highly-trained competitive athletes and amateur athletes in the effects caused by BJ supplementation could be a consequence of years of training adaptations and genetic factors [26].

The physiological mechanisms underlying the impacts of NO_3^- supplementation on cardiorespiratory endurance performance remain unclear. Studies have shown that factors such as NO_3^- dose, training level, athlete status, duration of supplementation (acute or chronic), regular dietary NO_3^- intake and exercise test duration and intensity may all affect the impacts of BJ consumption [12]. It remains clear that much further work is needed to elucidate the physiological adaptations and responses induced by BJ in trained or even untrained subjects before and after a training intervention [20].

In the studies performed to date, different test protocols have been used to assess the impacts of NO_3^- supplementation and there is some debate as to which tests are the most suitable for assessing endurance performance. Tests of time to exhaustion measure exercise capacity more than performance per se. These protocols have been criticized for their deficient ecological validity and their limited applicability to some sports modalities [27, 28]. Other studies have based their assessments on covering a given distance in the fastest time possible (time trial) as an intervention to improve sport performance [27, 29].

The arduous nature of laboratory cycling time trials means it is not possible to ask participants to execute a familiarization trial at the criterion distance, which could indicate a lack of knowledge of the performance variability of cyclists in time trials, especially in subjects not experienced in cycling [30]. Given the complexity of designing specific tests to simulate real sports conditions (lab tests vs races), we propose opting for a test conducted at an intensity equivalent to VT, in which aerobic performance (first ventilatory threshold or VT1) and transition towards an anaerobic energy metabolism (second ventilatory threshold or VT2) can be assessed. The use of submaximal VT workloads seems to more accurately predict cycling endurance performance [31]. This has been described as a valid method [32] that shows a direct relationship between VT and 40 km time trial performance. Moreover, the gas exchange threshold and VT are highly correlated with running velocity in triathlon and marathon tests [33–35], and VT2 is a strong predictor of performance in time-trials [36].

The objective of the present study was to assess the effects of acute BJ supplementation on endurance exercise performance and cardioventilatory responses in well-trained triathletes during a cycle ergometer test conducted at a load intensity equivalent to the first and second ventilatory threshold. Our working hypothesis was that a single dose of BJ supplementation would improve cardiorespiratory endurance performance by diminishing $\dot{V}O_2$ for a given workload by means of more efficient and economic mechanical and energy-producing physiological mechanisms.

Methods

Participants

Participants recruited were 12 well-trained triathletes at the national ($N = 8$) and international ($N = 4$) level (age, 39.3 ± 7.5 years; height, 176.5 ± 7.5 cm; weight, 72.8 ± 6.9 kg; BMI, 23.4 ± 2.2; VO_2max, 54.8 ± 3.1 mL·min^{-1}·Kg^{-1}) from different triathlon clubs in Madrid (Table 1). Participation was voluntary though we established the following inclusion criteria: a) national and/or international competition level; b) $VO_{2max} > 50$ mL·min^{-1}·kg^{-1} in cycling; c) no cardiovascular, respiratory, metabolic, neurological or orthopedic disorders that could affect cycle ergometer performance; d) no consumption of drugs or medication; e) no smoking; and f) no nutritional supplements taken in the three months before the study outset (e.g., caffeine, β-alanine, creatinine, sodium bicarbonate, glutamine, etc.). To be classified as well-trained, subjects had to have undergone training for at least 1 h at least 4 times per week and have competed in at least one organized cycle race in the preceding 12 months [30]. Sample size calculation was based on the results of a pilot study with the same study protocol involving 10 sport science students. The calculation of sample size was performed with α = 0.05 (5% chance of type I error) and 1- β = 0.80 (power 80%), and applying the results provided from previous studies, which used the same [17] or a smaller sample size [18]. A total of 12 well-trained triathletes was required for this study to detect differences between both experimental conditions.

The subjects were informed of the study goals and test protocols before giving their signed informed consent for participation. The study protocol received approval from the Ethics Committee of the Universidad Alfonso X El Sabio (Madrid, Spain).

Study design

Participants completed three cycle ergometer test sessions at our Exercise Physiology laboratory. As in previous studies [37, 38], a washout period of at least 72 h separated the laboratory visits. Sessions were conducted under the same ambient conditions (temperature 20 °C–22.5 °C, relative humidity 42–52%) and in the same time frame (± 0.5 h). Participants refrained from any high-intensity physical effort from 72 h and refrained from any type of physical exercise from 24 h before starting the first session to the study end. They were allowed to perform low intensity workouts, except 24 h before the start of the test.

Table 1 Descriptive characteristics of national and international triathletes

	National Level	International Level
Participants	$n = 8$	$n = 4$
Age (years)	39.4 (8.1)	39.0 (7.6)
Height (cm)	175.0 (7.3)	179.5 (8.1)
Weight (kg)	72.7 (5.1)	73.1 (10.8)
BMI	23.8 (2.4)	22.5 (1.6)
VO_2max (mL.kg^{-1}.min^{-1})	53.1 (2.1)	58.3 (1.7)

Data are provided as mean ± standard deviation (SD) Abbreviations used: *BMI* body mass index, VO_{2max} maximum oxygen uptake

In Session 1, an incremental test until exhaustion was performed on a cycle ergometer. In this test, determination was made of maximum or peak cardioventilatory indices and ventilatory thresholds VT1 and VT2. The power output (in W) eliciting VT1 and VT2 was recorded to determine the workload for the constant test at the intensity of VT1 and VT2 during sessions 2 and 3.

Sessions 2 and 3 were identical and both experimental conditions were compared BJ vs. placebo (PL). In these sessions, supplement assignment was double blind fashion and random. Participants took the supplement given to them, BJ or PL, as soon as they arrived at the lab ensuring that 50% of the triathletes randomly took PL in the first session and BJ in the second or vice versa. This meant that half the participants in each session worked under one of the two experimental conditions. Three hours after taking the supplement, the athletes started with a warm up before conducting an endurance test on a cycle ergometer at a constant workload (W) corresponding to VT1 (30 min) and, without rest, at a constant workload set at VT2 intensity (VT2 time trial) (~ 15 min). After VT2 time trial, participants answered a few questions to verify whether they were blinded to the supplementation condition. During 3-h period post BJ ingestion and before beginning the test, the triathletes remained under resting conditions.

Diet and supplementation

As an individual's diet can affect energy metabolism during exercise [39], subjects were given guidelines by a qualified nutrition professional to ensure that 48 h before each of the test sessions, they followed a similar diet consisting of ~ 60% carbohydrates (5.5 g carbohydrate per kg), 30% lipids and 10% proteins. Dietary ingestion was controlled during the 48-h period before each test session by means of a combination of usual diet and the nutritionist's recommendations. The diet consisted of typical food sources recommended for endurance athletes (e.g., bread, pasta, rice, milk, chicken, tuna, fruit, etc.) considering the energy intake from the PL and BR beverages.

The triathletes recorded their diet for the 48-h period before the first experimental test and replicated the same diet during the 48 h before the second trial. Upon arrival at the laboratory on a test day, participants' diaries were evaluated by a nutrition expert to determine compliance with established dietary instructions. In the case of not complying with the guidelines, the athlete was excluded from the study.

They were also provided with a list of foods with high NO_3^- contents they should avoid at least two days before the study outset (beetroot, celery, ruccula, lettuce, spinach, turnip, endives, leak, parsley, cabbage). Subjects were also instructed to avoid drinks containing caffeine or alcohol during the 24 h before the tests. No caffeine or alcohol intake was allowed during the study for avoiding any interaction with BJ.

Supplementation was given 3 h before the start of each test [19], as it has been established that NO_2^- peaks in blood 2–3 h after the intake of NO_3^- [40]. Each subject took the supplement by drinking the contents of a randomly assigned bottle containing 70 ml (~ 6.5 mmol, 404 mg of NO_3^-) of BJ concentrate Beet-It-Pro Elite Shot (Beet IT; James White Drinks Ltd., Ipswich, UK) or PL. The PL was a nitrate-depleted source and was prepared by dissolving 1 g of powdered BJ (~ 0.005 mmol, 0.311 mg of NO_3^-, ECO Saludviva, Alicante, Spain) in a litre of mineral water and adding lemon juice to imitate the taste of the commercial supplement. The PL supplementation was prepared by experts in nutrition and dietetics, and pharmacy. Both drinks (BJ and PL) were supplied in an unlabeled, 100-ml, brown glass bottle. During this period before the start of each test, the triathletes did not ingest food and fluids, apart from water, to guarantee hydration status.

Participants were asked to refrain from brushing their teeth or using a mouthwash, chewing gum or sweets that could contain a bactericidal substance such as chlorhexidine or xylitol in the 24 h prior to the test sessions. The reason for this is that the use of oral antiseptics can prevent increased blood NO_2^- levels after the intake of NO_3^- due to their effects on mouth bacteria [41].

All participants were warned of the possible side-effects of BJ, ie, gastrointestinal symptoms and the red appearance of urine and feces.

Cycle ergometer tests

We used an Ergoselect 200 cycle ergometer (Ergoline GmbH, Bitz, Germany) for the incremental and submaximal tests, that was calibrated and adjusted for use with the corresponding pedals and participants' footwear.

To measure the ventilatory variables, we used a gas analysis system (Ergostik, Geratherm Respiratory, Badd Kissingen, Germany) which was calibrated before each test using known O_2 and CO_2 concentrations and low, medium and high flow to calibrate ventilation. Gas exchange data were taken breath-by-breath to obtain the variables VO_{2max}, minute ventilation (VE), ventilatory equivalent for oxygen ($VE{\cdot}VO_2^{-1}$), ventilatory equivalent for carbon dioxide ($VE{\cdot}VCO_2^{-1}$), respiratory exchange ratio (RER), end-tidal partial pressure of oxygen and carbon dioxide ($PetO_2$ and $PetCO_2$ respectively). Heart rate was measured by telemetric recording using a transmitter fixed to the chest that sent data to a portable receiver (RS-800CX, Polar Electro OY; Kempele, Finland). Ventilatory and heart rate data were transferred to a PC for subsequent analysis.

Warm-up consisted of 5 min cycling at a light rhythm for the incremental test (Session 1), with subjects selecting the workload and cadence. Next, the triathletes started a ramp test until exhaustion with an initial 50 W load that was gradually increased in 25 W per minute (5 W every 12 s). The participants cycled at a self-selected pedal rate of between 70 to 90 rpm. The test was voluntarily terminated by the athletes when cadence dropped to below 70 rpm, or at the point of extenuation.

The VO_{2max} was taken as the highest 30-s mean value attained prior to exhaustion in the test [16]. After the test, the criteria used to determine VO_{2max} were [42]: (1) a plateau produced in the VO_2 curve with increases lower than 1.5 mL · kg^{-1} · min^{-1} between 30 s intervals; (2) RER above 1.10; and (3) a heart rate equal to or greater than the theoretical maximum. Maximum heart rate was recorded as the highest value obtained in the incremental test.

In addition to VO_{2max}, two investigators separately identified VT1 and VT2. If there was lack of agreement, the opinion of a third observer was sought. We defined VT1 as the workload at which increases were produced in both $VE{\cdot}VO_2^{-1}$ and $PetO_2$, without a concomitant increase in $VE{\cdot}VCO_2^{-1}$. Similarly, VT2 was determined when increases were produced in $VE{\cdot}VO_2^{-1}$ and $VE{\cdot}VCO_2^{-1}$, but this time accompanied by a drop in $PetCO_2$ [43, 44].

Sessions 2 and 3 were preceded by the same warm-up as for the incremental load test. The ensuing test protocol consisted of 30 min of pedaling at a freely selected rate between 70 and 90 rpm at a constant workload equivalent to VT1, plus a VT2 time trial (~ 15 min), to try to complete the whole test time of ~ 45 min. Ventilatory data were recorded as means at 30 s time intervals. The workload (in W) was selected for each individual from the VT1 and VT2 values determined in the incremental test.

The slow component of the exercise test was defined as the difference (Δ VO2, in mL · min^{-1}) between VO_2 at the end of exercise and VO_2 at the end of the third minute of exercise at a constant load, both at VT1 and VT2. The values for the end of minute 3 were taken as the mean of VO_2 from 2 min 40 s to 3 min 20 s, while those recorded at the end of exercise were the mean of the VO_2 values obtained for the last 2 min [45].

Mean cycling efficiency (CE) at VT1 and VT2 were expressed in W · L^{-1} · min^{-1} while gross mechanical efficiency (GE) was calculated as the ratio of work accomplished per minute (ie, W in kcal · min^{-1}) to energy consumed per minute (ie, in kcal · min^{-1}), as described

elsewhere [46]. Energy expenditure was calculated from VO_2 and the RER using the tables of Lusk (VO_2 L. min^{-1} · RER expressed in kcal · L^{-1} O_2) [47].

The following equations were used to calculate the rates of carbohydrate and fat oxidation [48]:

$$\text{Carbohydrate oxidation } \left(g \cdot min^{-1}\right) = 4.585 \cdot (VCO_2) - 3.226 \cdot (VO_2)$$

$$\text{Fat oxidation } \left(g \cdot min^{-1}\right) = 1.695 \cdot (VO_2) - 1.701 \cdot (VCO_2).$$

Blood lactate concentrations were measured in each participant by an experienced investigator using the analyzer Lactate ProTM 2 (Arkray Factory Inc., KDK Corporation, Shiga, Japan). Clean blood samples (5 μl) were obtained from the index finger of the left hand. Lactate measurements were made: 1) at rest, 2) 30 s before the end of the VT1 stage, and 3) at the end of the test (VT2).

Participants graded their fatigue using the subjective rating of perceived effort [49] at the same time points as the lactate determinations.

Statistical analysis

The Shapiro-Wilk test was used to check the normal distribution of the data, which are reported as mean and standard deviation (SD), mean and confidence intervals (95% CI) or percentage (%). A two-way ANOVA with repeated measures, supplement * intensity (BJ, PL * VT1, VT2, VT1 + VT2), was used to compare the effects of the two experimental conditions (BJ vs. PL) on the cardioventilatory, economy/efficiency, and metabolic variables during the constant-load test conducted at the intensity of VT1 and VT2. When appropriate, Greenhouse-Geisser probability levels were used to adjust for sphericity and Bonferroni adjustments were used to control for multiple post-hoc comparisons. A Student t-test for paired data was used to determine differences between BJ and PL. To determine the magnitude of the response to both experimental conditions (supplements) we estimated partial eta-squared (η_p^2). The scale for classification of η_p^2 was 0.01 = small, 0.06 = medium, 0.14 = large. We also calculated the probability of demonstrating the effectiveness of each supplement through statistical power (SP). Significance was set at $p < 0.05$. All statistical tests were performed using the software package SPSS version 19.0 for Macintosh (SPSS Inc., Chicago, IL, USA).

Results

Intake of BJ and PL supplementation was well tolerated by all participants of the study, however, some triathletes showed beeturia (red urine) and red stools. Participants ingested the prescribed dose of BJ and PL as determined by the nutritionist and their dietary interventions were consistent with established dietary guidelines. After the completion of the tests, all subjects were unable to differentiate between BJ and PL condition and, therefore, the triathletes were blinded to the supplementation condition.

Cardioventilatory responses and VO_2 kinetics

The cardioventilatory variables measured in the incremental test until exhaustion (Session 1) are shown in Table 2 and those recorded at VT1 and VT2 in the constant load tests are provided in Table 3.

No significant interaction effect (supplement*intensity) was observed on any of the cardioventilatory variables ($p > 0.05$). The only significant effect found was that of the supplement (BJ, PL) on VCO_2 ($F_{(1,\ 11)} = 20.155$, $p = 0.001$, $\eta_p^2 = 0.647$, SP = 0.983). No other effects of the supplement were noted ($p > 0.05$). Intensity effects were produced on heart rate ($F_{(2,\ 22)} = 89.325$, $p < 0.001$, $\eta_p^2 = 0.890$,SP = 1), VO_2 ($F_{(2,\ 22)} = 51.293$, $p < 0.001$, $\eta_p^2 = 0.823$, SP = 1), $\%VO_{2max}$ ($F_{(2,\ 20)} = 95.114$, $p < 0.001$, $\eta_p^2 = 0.905$, SP = 1), VCO_2 ($F_{(2,\ 22)} = 56.529$, $p < 0.001$, $\eta_p^2 = 0.837$, SP = 1), RER ($F_{(2,\ 22)} = 29.670$, $p < 0.001$, $\eta_p^2 = 0.730$, SP = 1), VE ($F_{(2,\ 22)} = 127.248$, $p < 0.001$, $\eta_p^2 = 0.920$, SP = 1), VE. VO_2^{-1} ($F_{(2,\ 22)} = 36.048$, $p < 0.001$, $\eta_p^2 = 0.766$, SP = 1), VE. VCO_2^{-1} ($F_{(2,\ 22)} = 22.244$, $p < 0.001$, $\eta_p^2 = 0.669$, SP = 1).

No significant impacts of the supplements (BJ vs PL) ($p > 0.05$) were detected on VO_2 kinetics measured through the slow component. A similar slow component was observed in both experimental conditions throughout the testing protocol in VT1 (BJ: 83 ± 45 mL. min^{-1}; PL: 71 ± 30 mL. min^{-1}) and in VT2 time trial (BJ: 227 ± 144 mL. min^{-1}; PL: 229 ± 129 mL. min^{-1}) (Fig. 1).

Table 2 Cardioventilatory parameters and load obtained in incremental test

Variable	VT1	VT2	VO_{2max}
Power (W)	195.4 (43.3)	282.1 (37.9)	390.3 (52.8)
VO_2 ($L{\cdot}min^{-1}$)	2.2 (0.4)	3.0 (0.3)	3.9 (0.5)
$VO_2{\cdot}Kg^{-1}$ ($mL{\cdot}min^{-1}{\cdot}Kg^{-1}$)	26.5 (11.9)	42.1 (4.6)	54.8 (3.1)
VCO_2 ($L{\cdot}min^{-1}$)	2.1 (0.5)	3.2 (0.4)	4.9 (0.8)
RER	0.9 (0.1)	1.1 (0.0)	1.3 (0.1)
V_E ($L{\cdot}min^{-1}$)	56.3 (10.7)	87.7 (14.2)	167.7 (36.5)
$V_E{\cdot}VO_2^{-1}$	24.7 (2.4)	28.1 (2.0)	41.2 (4.5)
$V_E{\cdot}VCO_2^{-1}$	26.8 (2.1)	26.8 (1.9)	32.3 (2.7)
$PetO_2$ (mmHg)	90.8 (4.2)	95.3 (3.1)	106.6 (2.8)
$PetCO_2$ (mmHg)	32.4 (2.9)	32.4 (2.6)	27.0 (2.4)
HR ($beats{\cdot}sec^{-1}$)	120.2 (13.5)	146.3 (13.1)	169.3 (12.8)
Intensity (% of VO_{2max})	55.8 (10.2)	75.7 (6.6)	–

Data are provided as mean ± standard deviation (SD) and percentage (%)
Abbreviations: HR heart rate, *$PETCO_2$* end-tidal partial pressure of carbon dioxide, *$PETO_2$* end-tidal partial pressure of oxygen, *RER* respiratory exchange ratio, *VCO_2* carbon dioxide, *VE* minute ventilation, *VE-VCO_2^{-1}* ventilatory equivalent for carbon dioxide, *VE-VO_2^{-1}* ventilatory equivalent for oxygen, *VO_2* oxygen uptake, *VO_{2max}* maximum oxygen uptake, *VT1* first ventilatory threshold, *VT2* second ventilatory threshold

Table 3 Comparison between beetroot juice (BJ) supplementation and placebo (PL) experimental condition on cardiorespiratory variables

	EC	VT1	VT2	Total (VT1 + VT2)	P^1	P^2
HR(beats·sec^{-1})	BJ	130.7 (17.3)	159.6 (11.7)	145.1 (13.8)	0.517	0.485
	PL	129.4 (17.2)	160.0 (14.0)	144.3 (14.0)		
VO_2 (L·min^{-1})	BJ	2.4 (0.4)	3.4 (0.3)	2.9 (0.3)	0.241	0.493
	PL	2.4 (0.5)	3.3 (0.4)	2.9 (0.4)		
VO_2 (%)	BJ	61.8 (11.3)	85.9 (7.6)	73.8 (8.9)	0.253	0.512
	PL	60.5 (12.2)	83.9 (8.3)	72.2 (9.4)		
VCO_2 (L·min^{-1})	BJ	2.5 (0.4)	3.7 (0.4)	3.1 (0.3)	**0.001**	0.579
	PL	2.4 (0.5)	3.5 (0.5)	2.9 (0.4)		
RER	BJ	0.9 (0.0)	1.1 (0.1)	1.0 (0.0)	0.106	0.623
	PL	0.9 (0.1)	1.1 (0.1)	1.0 (0.0)		
V_E (L·min^{-1})	BJ	74.9 (14.6)	127.6 (19.9)	101.2 (15.9)	0.054	0.622
	PL	72.9 (16.8)	125.5 (23.5)	96.8 (16.8)		
V_E·VO_2^{-1}	BJ	28.9 (2.1)	36.3 (4.5)	32.6 (2.9)	0.483	0.587
	PL	28.4 (1.9)	36.5 (5.7)	31.6 (2.5)		
V_E·VCO_2^{-1}	BJ	28.9 (1.6)	33.4 (3.1)	31.1 (2.1)	0.162	0.573
	PL	29.3 (1.6)	34.2 (4.4)	31.1 (1.9)		

Data are provided as mean ± standard deviation (SD) and percentage (%)
Abbreviations: EC experimental condition, *HR* heart rate, *RER* respiratory exchange ratio, *VCO_2* carbon dioxide, *VE* minute ventilation, *VE·VCO_2^{-1}* ventilatory equivalent for carbon dioxide, *VE·VO_2^{-1}* ventilatory equivalent for oxygen, *VO_2* oxygen uptake, *VO_{2max}* maximal oxygen uptake, *VT1* first ventilatory threshold, *VT2* second ventilatory threshold
[1]Significant differences for supplementation effect
[2]Significant differences for supplementation x intensity interaction

Cycling efficiency, gross mechanical efficiency, lactate, VT2 time trial

The CE, GE and VT2 time trial data are shown in Fig. 2. As occurred for the cardioventilatory variables, there were no significant interaction (supplement*intensity) effects on CE, GE or VT2 time trial ($p > 0.05$). Neither was a significant supplement effect produced on any of the variables ($p > 0.05$). However, as expected, significant intensity effects were produced on CE ($F_{(2,\ 22)} = 12.824$, $p < 0.001$, $\eta_p^2 = 0.538$, SP = 0.992), GE ($F_{(2,\ 22)} = 6.495$, $p < 0.001$, $\eta_p^2 = 0.733$, SP = 1), lactate ($F_{(2,\ 18)} = 24.743$, $p < 0.001$, $\eta_p^2 = 0.733$, SP = 1), and on VT2 time trial ($F_{(2,\ 20)} = 95.114$, $p < 0.001$, $\eta_p^2 = 0.905$, SP = 1).

Although no significant differences ($p > 0.05$) were found between BJ and PL in VT2 time trial and VT1 + VT2 (Fig. 1d), BJ supplementation lead to a shorter VT2 time trial (BJ: 15 min 33 s, PL: 14 min 42 s).

Energy expenditure, carbohydrate oxidation, fat oxidation and RPE

Data related to energy expenditure, carbohydrate oxidation and fat oxidation are shown in Fig. 3. No significant interaction or supplement effects on any variable were produced ($p > 0.05$). Significant intensity effects were observed on carbohydrate oxidation ($F_{(2,\ 22)} = 81.339$, $p < 0.001$, $\eta_p^2 = 0.881$, SP = 1), and energy expenditure ($F_{(2,\ 20)} = 91.043$, $p < 0.001$, $\eta_p^2 = 0.901$, SP = 1). No significant intensity effect was detected on fat oxidation ($p > 0.05$). No significant interaction or supplement effects were produced on RPE ($p > 0.05$).

Discussion

As far as we know, this is the first study to examine the possible effects of acute BJ supplementation on a constant workload cycloergometry exercise conducted at VT1 + VT2 time trial in well-trained endurance triathletes. As VT seems to be the most accurate predictor of endurance performance, especially in cycling [31], this study was designed to test the efficacy of BJ at improving performance during aerobic energy metabolism (VT1) and during the transition from aerobic to anaerobic metabolism (VT2).

Contrary to our working hypothesis, acute BJ supplementation was not observed to improve cardioventilatory responses, mechanical exercise economy/efficiency, slow component, use of energy substrates or performance in these athletes. Even though national athletes were less trained than international athletes, no positive effect of BJ supplementation was observed in both international and national athletes.

Our VT1 data confirm the results of other studies [50, 51] in which neither were improvements observed in cardioventilatory responses to low-moderate intensity submaximal exercise after supplementation with NO_3^-. Cristensen et al.

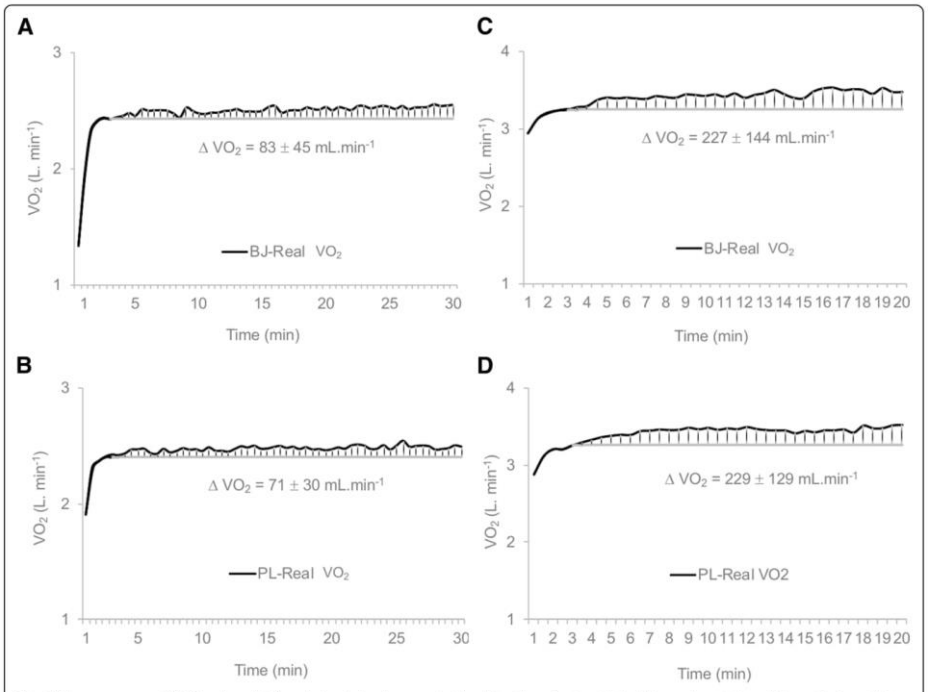

Fig. 1 Slow component (ΔVO_2 mL . min^{-1}) analysis during the constant load test in: **a** Beetroot juice (J) experimental condition at first ventilatory threshold (VT1); **b** Placebo (PL) experimental condition at VT1 **c** Beetroot juice (BJ) experimental condition at second ventilatory threshold (VT2); **d** PL experimental condition at VT2. Data are provided as mean and standard deviation. There were no significant differences between both experimental conditions (BJ vs. PL) in VT1 and VT2

(2013) [20] reported no GE increase after the intake of 0.5 L of BJ over 6-day periods (0.5 g nitrate per day). Their test protocol involved different work types including work and rest periods in elite cyclists. In another study, Bescos et al. (2011) [50] also detected no GE improvements in well-trained cyclists and triathletes in response to acute sodium nitrate supplementation (10 mg · kg^{-1} dissolved in 250 mL of water), in a test in which there was a single transition at different intensities and with a limited rest period. In contrast, others have shown increases in GE [51] and reductions in pulmonary VO_2 and O_2 cost in submaximal low-moderate intensity exercise in healthy moderately- and well-trained athletes following the intake of BJ [14] (0.5 L for 6 days, 5.5 mmol per day of NO_3^-) and sodium nitrate (0.1 mmol kg^{-1} bodyweight day^{-1}) [51] using different supplementation protocols and cycle ergometry as the assessment test. There is no consensus on the appropriate dose in well-trained athletes at low-moderate exercise intensity.

Previous studies have demonstrated that higher BJ supplementation dose (~ 8.4 mmol and ~ 16.8 mmol of NO_3^-) caused a greater reduction in systolic blood pressure and mean arterial pressure at moderate exercise intensity than lower doses (~ 4.2 mmol of NO_3^-) in healthy adults [37]. In this study, VO_2 steady-state of moderate exercise intensity was reduced significantly after ingestion of 16.8 mmol of NO_3^-, tended to be lower after intake of 8.4 mmol NO_3^-, and was unaffected by 4.2 mmol of NO_3^- [37]. Higher doses of BJ supplementation (2 × 70 mL doses per day, ~ 6.2 mmol of NO_3^- per 70 mL) before and during prolonged moderate-intensity exercise might be necessary to attenuate the progressive rise in VO_2 and reduce muscle glycogen depletion [52], improving mechanical efficiency during a prolonged constant-load test at VT1 intensity. Further, IOC consensus statement concludes that longer periods (> 3 days) of NO_3^- supplementation could increase sport performance in highly-trained athletes [22].

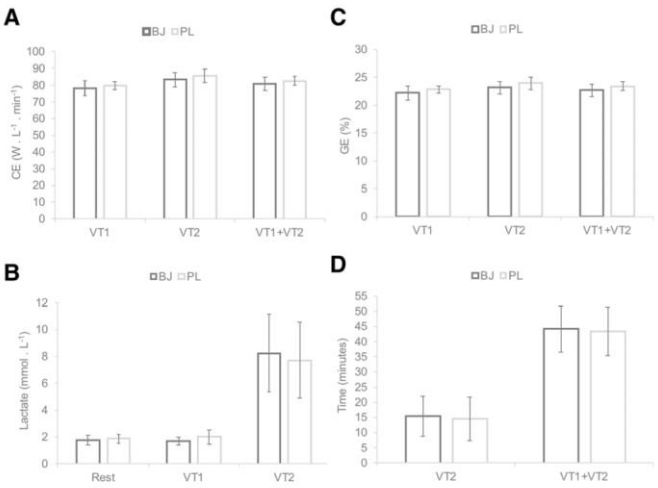

Fig. 2 Differences between beetroot juice (BJ) and placebo (PL) at first ventilatory threshold (VT1), second ventilatory threshold (VT2) and in the total time of the test (VT1 + VT2), measured in: **a** Cycling efficiency (CE); **b** Gross efficiency (GE); **c** Lactate; **d** Total time until exhaustion. Data are provided as mean and error bars as 95% confidence intervals. There were no significant differences between both experimental conditions BJ vs. PL

It is unclear that dose-response relationship exists between acute BJ supplementation and the physiological mechanisms for the reduction in the O_2 cost and pulmonary O_2, decrease in VO_2 slow component and increases in GE during low-moderate exercise intensity. Probably, the dose used in our study was not enough to cause an ergogenic effect in well-trained triathletes.

The type and/or mode of supplementation or the test used do not seem to play as important a role as cardiovascular fitness level when assessing VO_2, O_2 cost and GE at low-moderate intensity, as moderately-trained healthy athletes have shown a favorable response to BJ supplementation as opposed to a negative response of well-trained athletes, regardless of the test or supplementation protocol (acute or chronic). Subjects with a lower fitness level may be more susceptible to BJ effects regardless of whether the BJ supplementation is acute or chronic. In effect, the literature suggests some interaction between training state and the ergogenic effects of NO_3^- supplements [53], though the physiological mechanisms induced remain unclear and are likely related to adaptations achieved in response to endurance training [54]. It is known that the most skilled individuals feature better vascular control, characterized by a greater activity and presence of the enzyme endothelial nitric oxide synthase (eNOS), responsible for endogenous NO production [55]. Thus, any increase in eNOS activity could reduce the availability of NO derived from nitrates, consequently diminishing the possible effects of BJ. This rationale could explain, at least in part, the results obtained here for VT1 as this is a low-moderate intensity of exercise after which a first evident shift is produced in ventilation and in blood lactate concentrations and above which anaerobic energy metabolism is partly involved [56]. Lactate concentrations in our athletes at VT1 were lower than 2 mmol. L^{-1} indicating a predominantly aerobic state. During this metabolic stage, such intensity of exercise may be maintained over a long period of time without marked changes in blood lactate concentrations [57, 58]. Hence, it is less likely that a trained athlete will experience low muscle oxygenation increasing muscle acidosis and generating nitrate reduction at a given work rate [30]. We suspect there was no tangible effect of BJ, as more trained subjects could show reduced O_2 uptake due to a decrease in the aerobic energy required or in the muscular energy used in moderate exercise efforts.

The reduction in VO_2, attributed to reduced ATP resynthesis through oxidative phosphorylation, was not offset by elevated glycolytic ATP provision [14], as indicated by the similar blood lactate concentrations observed in the groups BJ and PL. However, as argued by Bailey et al. (2009) [14], in less trained subjects, a beneficial effect of NO_3^- is produced reflected by increased muscle oxygenation indices and total hemoglobin levels during moderate exercise. The increased blood volume

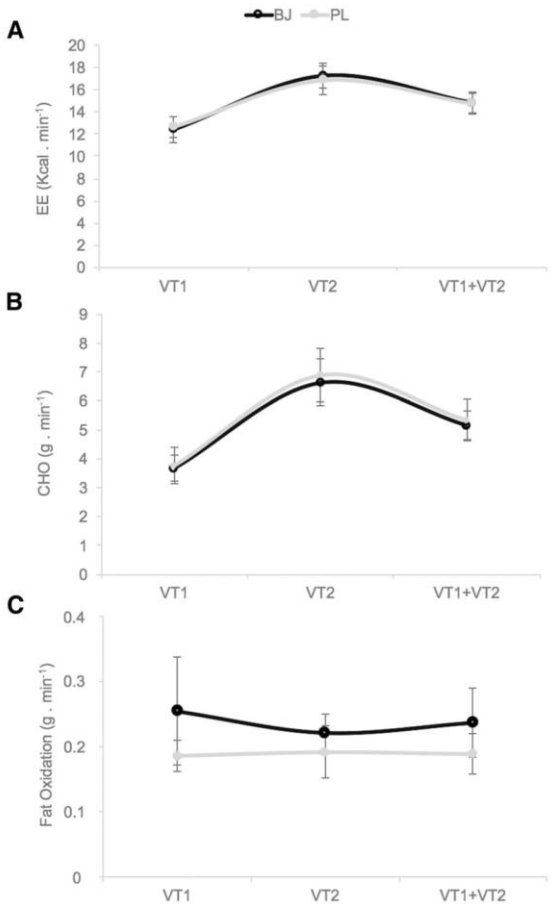

Fig. 3 Differences between beetroot juice (BJ) and placebo (PL) at first ventilatory threshold (VT1), second ventilatory threshold (VT2) and in the time to completion (VT1 + VT2), measured as: **a** Energy expenditure (EE); **b** Carbohydrate oxidation (CHO); **c** Fat oxidation. Data are provided as mean and error bars as 95% confidence intervals. There were no significant differences between both experimental conditions BJ vs. PL

observed in the vastus lateralis muscle after BJ intake is presumably a consequence of improved muscular vasodilation resulting from the increased production of NO from NO_2^-.

Compared to VT1, physiological and efficiency (intensity effect) changes were observed here in VT2. Studies have shown that BJ enhances high-intensity endurance exercise performance in moderately-trained subjects [14, 59] while its effects are not so clear in well-trained subjects. In a study conducted in elite cyclists [20], the time taken to complete a time trial failed to vary significantly between individuals given BJ or PL. This is similar to the effects on VT2 observed in the present study (400 kcal-time trial 18:20; VT2 time trial 15:33 min:s, respectively), with comparable levels of power reached (290.0 ± 43.0 W vs 282.1 ± 37.9 W respectively). Thus, it could be that the intensities set in both tests (preload vs. VT1 and 400 kcal-time trial vs. VT2 time trial) gave rise to an aerobic metabolism and transition to an anaerobic energy pathway. In statistical terms, BJ showed no endurance performance-enhancing

effect in both studies (0.8% and 5.7% in our study). However, significant performance improvements in response to BJ have been observed in well-trained cyclists and triathletes of 1.2% [17] and in rowers (− 1.6 ± 1.6 s) [23], along with an increase, though not significant, of ~ 2% in trained cyclists and athletes [50], and a beneficial response in some elite athletes [60]. Collectively, these findings point to a possible ergogenic effect of BJ on the cardiorespiratory performance of highly-trained endurance sport athletes. It should be considered that to increase the possibility of winning, a high-level endurance sport athlete needs to achieve a gain in total time of at least 0.6% [61]. For example, the variance between twelfth and first place in the 10,000 m men's running final at the 2012 London Olympics was only 0.66% [62]. Such a slight biological improvement induced by BJ supplementation (not statistically significant), together with intrinsic and extrinsic motivational factors, could be determining factors for success in high-level athletes and this impact may have been detectable in a larger sample size. It would be logical to assume that BJ supplementation could at least partly influence cardiorespiratory performance especially when small improvements in endurance tests can be particularly meaningful. Because these changes in performance are so small, it would be noteworthy to evaluate the differences between physiological and motivational factors produced by BJ supplementation.

Currently, the scientific literature lacks data on the effects of nitrates on high-intensity exercise [53] and the assumption gains importance that BJ supplementation could improve the capacity to cope with fatigue in situations of transition from an aerobic to anaerobic energy metabolism, despite a poor understanding of the physiological etiology involved in well-trained athletes. This is especially true as the slight, yet interesting, increase in VT2 time trial took place in the absence of a beneficial impact of BJ on the cardioventilatory response, exercise economy/efficiency, slow component, use of substrates and blood lactate concentrations. Maybe, an increase in the BJ supplementation dose would have been a factor key to detect improvements in the variables analyzed in our study. Previous findings have shown that higher BJ supplementation dose (~ 8.4 mmol and ~ 16.8 mmol of NO_3^-) improves the time-to-task failure when is compared with a 4.2 mmol dose in young healthy adults [37]. Effectively, in well-trained subjects it would be necessary to intake larger NO_3^- doses (140 mL, ~ 8.4 mmol, 550 mg of NO_3^-) [23]. A normal dose (70 mL) is unlikely to trigger an ergogenic effect. Higher dose (~ 16.8 mmol of NO_3^-) raises the plasma NO_2^- levels to a greater extent than ~ 8.4 mmol of NO_3^-, however, no added performance gains are produced [23]. Prolonged periods of BJ supplementation longer than 3 days could increase sports performance [24, 25] and could be used as an alternative supplementation strategy for well-trained athletes.

Furthermore, whole vegetables have been demonstrated to provide important health benefits whereas NO_3^- from other sources could lead to adverse effects on health [63]. Because NO_3^- consumed in the form of vegetables have been shown to improve running performance in healthy adults [63], it is tempting to speculate that supplementation strategy based on whole beetroot could be an interesting choice for well-trained competitive athletes while preserving their health. More studies analysing the effects of whole vegetables intake on endurance performance in well-trained athletes are necessary to substantiate such claims.

As a final remark, changes in exercise intensity from VT1 to VT2 involve variations in VO_2, leading to the use of different substrates. Carbohydrates are more efficient as energy substrates than fatty acids. In other words, if more carbohydrates are used as substrate this gives rise to lower oxygen absorption at a given work velocity [51]. Calculations of GE include possible RER changes and, therefore, take into account substrate use. Neither did BJ seem to induce more efficient substrate use as reflected by our data for GE, RER, and consumption of energy, carbohydrates and fats in both experimental groups. Further, our GE calculations were targeted at assessing the effects of blood alkalization on gradual losses in muscle efficiency as the best indicator of the so-called slow component phenomenon [64]. With this protocol inducing a change in metabolism from VT1 to VT2, we sought to examine the effects of BJ after promoting a change in VO_2 kinetics (slow component). This change is similar to that observed after an initial bout of high-intensity exercise, giving rise to increased muscle O_2 release, increased oxidative metabolic enzyme activity, carbon substrate availability, and abnormal motor unit recruitment patterns [65, 66]. It is not clear in the scientific literature whether any of these physiological mechanisms could reduce the slow component in response to BJ supplementation in healthy moderately-trained subjects [14, 67]. In a recent study, Tan et al. (2018) [52] demonstrated that BJ supplementation mitigated the progressive rise in VO_2 over time before and during prolonged moderate-intensity exercise although did not enhance subsequent time trial performance. Interestingly, it was observed that this decrease in VO_2 had no impact on time trial performance, which could indicate that supplementation with BJ does not sufficiently reduce muscle glycogen depletion at moderate intensity for decreasing fatigue during cycling time-trial. More research is needed to analyze the BJ supplementation effect on VO_2 kinetics during endurance tests over two hours.

There are some limitations in this study which should be considered. Previous research indicates that the plasma nitrite of the participants should increase to show an ergogenic effect, however, nitrite and nitrate concentrations in

plasma were not measured in our study. It seems that doses close to or greater than 8.4 mmol are more adequate to determine the positive effects of BJ supplementation on endurance performance in well-trained triathletes [23].

The small sample size in this study should be taken into account when drawing conclusions from the data. Minimal changes in endurance performance are usually observed in well-trained triathletes, therefore, large sample sizes should be required to detect significant changes produced by BJ supplementation on cardioventilatory performance.

Although there are several studies that have carried out a similar washout period, it is possible that the washout period established in our study was not sufficient, which could influence the final results.

Conclusions

Our findings do not support an improvement in the variables examined here produced in response to acute BJ supplementation. We have yet to elucidate the possible ergogenic effects of BJ in highly trained athletes. However, the slight (not significant) modifications observed in performance variables such as test duration or maintaining work intensity at a given load in several studies prompts numerous questions as the mechanical and physiological mechanisms analyzed so far do not support these improvements and remain poorly understood.

Our outcomes suggest a need to analyze individual positive responses to this form of supplementation in well-trained athletes.

Abbreviations

ANOVA: Analysis of variance; BMI: Body mass index; CE: Cycling efficiency; CI: Confidence intervals; GE: Gross mechanical efficiency; NO: Nitric oxid; NO_2^-: Nitrite; NO_3^-: Nitrate; $PetCO_2$: End-tidal partial pressure of carbon dioxide; $PetO_2$: End-tidal partial pressure of oxygen; PL: Placebo; RER: Respiratory exchange ratio; RPE: Rating of perceived exertion; rpm: Revolutions per minute; SP: Statistical power; VE: Minute ventilation; $VE{\cdot}VCO_2^{-1}$: Ventilatory equivalent for carbon dioxide; $VE{\cdot}VO_2^{-1}$: Ventilatory equivalent for oxygen; VO_2: Oxygen uptake; VO_2max: Maximum oxygen uptake; VT1: First ventilatory threshold; VT2: Second ventilatory threshold; W: Watt; η_p^2: Partial eta-squared

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Availability of data and materials

Data are presented in the manuscript, further information available upon request.

Authors' contributions

RD, JLM-M and MVG-C conceived and designed the experiments; RD, GP-S, EC, PG-F, AM-G, MCL-E and MVG-C performed the experiments; RD, GP-S, MVG-C, PV-H and JLM-M analyzed the data; AM-G, EC, PG-F, and MCL-E contributed reagents/materials/analysis tools; RD, JLM-M, and MVG-C wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The subjects were informed of the study goals and test protocols before giving their signed informed consent for participation. The study protocol received approval from the Ethics Committee of the Universidad Alfonso X El Sabio (Madrid, Spain) according to the principles and policies of the Declaration of Helsinki.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Effects of Beetroot Juice Supplementation on a 30-s High-Intensity Inertial Cycle Ergometer Test

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Abstract: Background: Beetroot juice (BJ) is rich in inorganic nitrates and has proved effective at increasing blood nitric oxide (NO) levels. When used as a supplement BJ has shown an ergogenic effect on cardiorespiratory resistance exercise modalities, yet few studies have examined its impact on high intensity efforts. **Objective:** To assess the effects of BJ intake on anaerobic performance in a Wingate test. **Methods:** Fifteen trained men (age 21.46 ± 1.72 years, height 1.78 ± 0.07 cm and weight 76.90 ± 8.67 kg) undertook a 30-s maximum intensity test on an inertial cycle ergometer after drinking 70 mL of BJ (5.6 mmol NO_3^-) or placebo. **Results:** Despite no impacts of BJ on the mean power recorded during the test, improvements were produced in peak power (6%) ($p = 0.034$), average power 0–15 s (6.7%) ($p = 0.048$) and final blood lactate levels (82.6%) ($p < 0.001$), and there was a trend towards a shorter time taken to attain peak power (−8.4%) ($p = 0.055$). **Conclusions:** Supplementation with BJ has an ergonomic effect on maximum power output and on average power during the first 15 s of a 30-s maximum intensity inertial cycle ergometer test.

Keywords: beet; nitrate; physical activity; sport; supplement

1. Introduction

Beetroot juice (BJ) is a source of inorganic nitrate (NO_3^-) found in other vegetables or used as preservatives for processed meat products [1]. After the intake of BJ, around 25% of the NO_3^- present is reduced by bacteria in the mouth to nitrite (NO_2^-). As it reaches the stomach, some of this NO_2^- is reduced to nitric oxide (NO) [2], and subsequently absorbed along with the nonreduced nitrite in the gut passing into the bloodstream [3] where blood NO and NO_2^- concentrations rise. Besides this rise in NO levels produced after consuming NO_3^- [4], in situations of low oxygen levels the NO_2^- present in blood may be again reduced to NO [3]. Thus, the final result of taking a BJ supplement is that blood NO levels rise.

NO plays a key role in several physiological, hemodynamic and metabolic events [5]. NO causes blood vessel dilation through mediation by guanylate cyclase [6], increasing blood flow to the muscles and reducing VO_2 at a given work rate [7]. Studies have indeed shown that NO has beneficial effects on muscle contraction [8] and biogenesis [9] and mitochondrial efficiency [10]. Nitric oxide plays a role in efforts that require an oxidative-type of energy metabolism as in endurance exercises performed

at a work rate lower than VO_{2max} and of duration longer than 5 min [11]. In these high-intensity efforts, many studies—though not all [12–16]—have measured performance or endurance indicators such as economy following the intake of BJ [17–28]. Hence, in endurance exercise modalities, BJ supplementation has been reported to reduce VO_2 at work rates equivalent to the ventilatory threshold (VT) [10], first lactate threshold (LT1), second lactate threshold (LT2) [26], 45% VO_{2max} [18], 50% VO_{2max} [25], 60% VO_{2max} in conditions of normal oxygen levels [21] and low levels [24,28], 65% VO_{2max} [18] and 70% VO_{2max} [25,28]. In addition, BJ supplementation has shown an ergogenic effect in cycle ergometry tests until exhaustion executed at work rates equivalent to 60% VO_{2max}, 70% VO_{2max}, 80% VO_{2max} [13], 90% VO_{2max} [25] and to 70% [20] or 75% [22] between VT and VO_{2max}, as well as improved performance at 4- [29], 10- [23] and 16-km tests in normoxia [17] and hypoxia [24], 50 miles in normoxia [19] and of 30 min in hypoxia [27].

Apart from endurance efforts, other sport modalities exist in which the predominant energy metabolism, rather than involve oxidative energy processes, entails pathways that are independent of oxygen as is the case for explosive or high intensity efforts [30]. Explosive efforts are those lasting under 6 s in which the main energy metabolism pathway is the high-energy phosphagen system and there is some participation also of glycolysis and oxidative phosphorylation [31]. This pathway gradually contributes more to energy production until it accounts for 50% of this at 6 s [31]. High-intensity efforts are those of duration 6 to 60 s that feature a major contribution of glycolytic metabolism and smaller participation of high-energy phosphagens and oxidative phosphorylation [30]. Compared to endurance efforts, these high intensity efforts potentially have an even greater capacity to increase blood NO concentrations in response to BJ supplementation. This is because during the execution of this type of exercise movement, in which the main energy metabolism is independent of oxidation reactions, a drop is produced in the partial pressure of oxygen and pH in muscle and venous and capillary blood [32], and these conditions promote the reduction of NO_2^- to NO [3].

Studies in animals have shown that NO's blood flow improving effect is greater for type II than type I motor units [5,29]. Further, also in animals it has been noted that the power production improvement produced in response to BJ is specific to motor type II units [33]. This is because this type of muscle unit has a greater power production capacity and is designed to obtain energy via non-oxidative pathways. This could be due to the greater capacity of these units to store glycogen and muscular creatine [34], as well as proteins such as carnosine [35], which have a buffering effect at the intracellular level [36]. Thus, BJ intake could have an ergogenic effect during both explosive efforts and high intensity efforts. A 30-s maximum sprint test on a cycle ergometer (Wingate test) can be used to assess performance at high intensity efforts by determining power output and glycolytic capacity [37]. In addition, explosive efforts can be assessed in the first 5 s of the Wingate test, as in this interval adenosine triphosphate (ATP) resynthesis occurs mainly via the high-energy phosphagen system [38]. Accordingly, in the present study, we examined the effects of BJ supplementation on anaerobic performance in a Wingate test conducted by athletes trained in sports modalities with a high glycolytic energy metabolism component.

2. Materials and Methods

2.1. Participants

Participants were 15 male undergraduates of Physical Activity and Sport Sciences with experience with the Wingate test (they had performed at least one test in the month before the study onset). Descriptive data for the study population are provided in Table 1. Participation in the study was voluntary, though subjects were required to fulfil the following inclusion criteria: (a) more than two years' experience in sports modalities with a high glycolytic energy metabolism component (speed tests in athletic sports and swimming, combat and team sports); (b) not considered an elite athlete; (c) an absence of cardiovascular, lung, metabolic, or neurologic disease or of an orthopaedic disorder

that could limit cycle ergometry performance; (d) no medication; (e) no smoking; (f) no nutritional supplements in the six months prior to the study onset.

Table 1. Characteristics of the 15 study participants.

Variable	M ± SD
Age (years)	21.46 ± 1.72
Height (cm)	1.78 ± 0.07
Weight (kg)	76.90 ± 8.67
BMI (kg/m^2)	24.21 ± 1.72
Kilogram-force (Kp)	5.77 ± 0.64

BMI = body mass index; M ± SD = mean (±standard deviation).

The subjects recruited were asked to attend a meeting the week before the study outset. In this meeting, three investigators informed them of the study protocol and gave them instructions about diet control and resolved any concerns they had. At the end of the meeting, they all signed an informed consent form. The study protocol was approved by the Ethics Committee of the Universidad Alfonso X El Sabio, Madrid, Spain (code number 1.010.704).

2.2. Study Design

Participants attended two testing sessions at the Exercise Physiology lab within the same time frame (±0.5 h) 72 h apart. From 72 h before the first session until the end of the study, subjects undertook no type of physical exercise. As soon as they arrived at the laboratory, in a random and double-blind fashion, subjects were given a BJ or placebo supplement ensuring that 50% of the subjects randomly took BJ in the first session and placebo in the second or vice versa. This meant that half the subjects in each session worked under one of the two experimental conditions. Three hours after intake of the supplement, subjects started a Wingate cycle ergometer test session including a warm-up.

2.3. Nutritional Intervention and Dietary Control

As the blood NO_2^- peak occurs 2–3 h post-ingestion, the supplement was administered 3 h before the endurance test [11]. The use of oral antiseptics can prevent increased blood NO_2^- levels after the intake of NO_3^- because of their bactericidal effect on the bacteria in the mouth. Thus, participants were asked to refrain from brushing their teeth or using a mouthwash, chewing gum or sweets that could contain a bactericidal substance such as chlorhexidine or xylitol in the 24 h prior to the test sessions.

Subjects were also instructed to avoid drinks containing caffeine during these 24 h due to its ergogenic effect [39]. The intake of alcohol was also restricted the day before the study start.

As an individual's diet can affect energy metabolism during exercise, subjects were given guidelines to ensure that 48 h before each of the test sessions, they followed a similar diet consisting of 60% carbohydrates, 30% lipids and 10% proteins and avoiding foods with high NO_3^- contents (beetroot, celery, arugula, lettuce, spinach, turnip, endives, leak, parsley, cabbage). Participants were provided with a list of vegetables they should avoid the day before the study outset.

Each subject randomly took the supplement by drinking the contents of a randomly assigned bottle containing 70 mL of BJ concentrate Beet-It-Pro Elite Shot (Beet IT; James White Drinks Ltd., Ipswich, UK) or placebo. The placebo was prepared by dissolving 1 g of powdered BJ (ECO Saludviva, Alicante, Spain) in a litre of mineral water and adding lemon juice to imitate the taste of the commercial supplement. Although the beetroot juice present in the placebo could have a minimum content of NO_3^-, the small proportion of desiccated beetroot juice in each bottle of placebo (0.015 g), along with the restricted intake of foods rich in NO_3^- 48 h before the start of each session ensured that subjects working under the placebo condition were depleted of NO_3^-.

Both drinks (BJ and placebo) were supplied in an unlabeled, 100-mL, brown glass bottle.

All participants were warned of the possible side-effects of BJ: gastrointestinal problems and the red appearance of urine and faeces.

2.4. Wingate Test

The Wingate test was started with the subject stopped. Before the test, the following instructions were given by the investigators: (i) in the first seconds of the test, they should pedal from 0 rpm to the greatest pedalling velocity possible (rpm) in the shortest time possible; and (ii) maintain this high power level during the longest time possible until the test end.

For the test, a Monark cycle ergometer (Ergomedic 828E, Vansbro, Sweden) was used. This ergometer consists of a metal wheel with a band which, through friction, offers resistance to pedalling. This resistance may be regulated as the band is connected to a pendulum that presses on it and this pressure is modified by adjusting a screw under the handle bar. Vertical elevation of the pendulum indicates the kilograms (kg) of friction exerted on the wheel. This friction of the band against the wheel is measured in kilogram force (kgf) defined as the force acting on a 1-kg mass subjected to the acceleration of gravity.

The Monark cycle ergometer has a cog of 52 teeth and a pinion of 14 teeth, causing the conversion of 3.71 revolutions of the wheel for each complete circle of the pedals. The wheel perimeter is 1.62 m and, as for each pedal the wheel spins 3.71 times, the wheel covers 6 m for each complete pedal revolution. The revolutions per minute (rpm) are counted by a magnet system on the pedalling axel and indicated on the speedometer. To calculate the power exerted on the pedals we need to multiply force by the movement velocity:

$$\text{Power} = \text{Force on the pendulum (kgf)} \times \text{Pedalling velocity (rpm)}$$

The force exerted by the band friction is read on the pendulum and expressed in kgf. Velocity is obtained multiplying the rpm of the pedals by the wheel's revolutions. When we multiply the kgf by the metres covered per minute we get kilopondmetres (kpm). To convert kpm into watts (W) one has to divide by 6.12:

$$\text{Kilopondmetres to watts} = 6.12 \text{ kpm} = 1 \text{ watt}$$

As for the Monark cycle ergometer, each complete pedal circle makes the wheel advance 6 m, each rpm is equivalent to 6 m/min. Thus, using this cycle ergometer, by multiplying the rpm by the kgf indicated by the pendulum, we obtain as a result the power in watts. For data extraction, the display of the Monark cycle ergometer was recorded with a video camera where the rpm during the whole test appeared. Subsequently, the video recording was transferred to the program Kinovea (version 0.8.15, France) which reproduces 30 photo frame/s and the rpm where compiled for each second. Next, we used the equation to determine the watts generated in each second of the test.

Subjects first performed a 5-min warm-up consisting of light cycling with the workload and cadence set by the subject followed by 1 min of rest. After this rest period, subjects executed a specific warm up of 3 min of pedalling at a rate of 60 rpm with a workload of 2 kgf and a sprint at maximum intensity in the last 5 s of each minute. After 3 min of rest, the Wingate test was started.

The test consisted of 30 s of cycling at maximum effort with a load (kgf) corresponding to 7.5% of the subject's body weight [40]. Participants were instructed to pedal as fast as possible to reach the maximum rpm in the shortest time possible and to try to maintain this pedalling speed until the end of the test. Two of the authors motivated the subjects during the test duration. As soon as the test was completed, the subjective rate of perceived exertion (RPE) scale used to rate leg muscle, cardiorespiratory and general perceived exertion.

Just before the warm up and 3 min after the test end, an examiner took a finger prick blood sample (5 µL) from the left index finger for blood lactate determination using a Lactate ProTM 2 LT-1710 blood analyzer (Arkray Factory Inc., KDK Corporation, Shiga, Japan).

Besides blood lactate and muscle, cardiorespiratory and general RPE, the power (W) variables obtained in the test were analyzed through their transformation of the product of rpm × kgf at W. In this way, the variables of W for each second were examined, obtaining cutoffs for 5-s, 10-s and 30-s intervals during the course of the test. Further variables recorded were: peak power (W_{peak}), time-to-W_{peak}, minimum W (W_{min}), mean power and fatigue index (($W_{peak} - W_{min}$)/W_{peak} × 100).

2.5. Statistical Analysis

Initially we confirmed the normal distribution of the data using the Shapiro–Wilk test.

The Student *t*-test for related samples was used to compare the performance variables recorded for the two experimental conditions (placebo and BJ).

All data are provided as the mean (M) and standard deviation (SD). All statistical tests were performed using the software package SPSS version 19.0 (SPSS, Chicago, IL, USA).

3. Results

Capillary blood lactate levels recorded before (resting lactate) and after the Wingate test (final lactate) and RPE scores after the test are provided in Table 2. The only significant difference detected was an 82.6% higher final lactate level in the group of subjects who took BJ supplements ($p < 0.05$).

Table 2. Metabolic variables and rating of perceived effort recorded in response to the Wingate test according to the experimental conditions (beetroot juice or placebo supplementation).

Variables	Placebo	CV (%)	BJ	CV (%)	%	*T*	*p*
Lactate-resting (mmol·L^{-1})	1.7 ± 0.45	26.6	2.0 ± 0.53	26.7	15.9	−2.051	0.059
Lactate-final (mmol·L^{-1}) *	7.4 ± 2.84	38.0	13.6 ± 4.12	30.2	82.6	−5.337	0.000
RPE-muscular	17.33 ± 1.58	9.2	17.80 ± 1.14	6.4	2.7	−1.388	0.187
RPE-cardiovascular	16.53 ± 2.50	15.1	16.73 ± 1.70	10.2	1.2	−0.315	0.757
RPE-general	17.60 ± 1.88	10.7	17.86 ± 1.12	6.3	1.5	−0.459	0.653

BJ: beetroot juice; RPE = rating of perceived exertion; CV = coefficient of variation; * significant difference for placebo vs. BJ ($p < 0.05$). Data provided as the mean and standard deviation.

The power variables recorded in the Wingate test are shown in Table 3. These data revealed that despite no differences in mean power ($p = 0.796$) between the two supplementation groups, peak power was significantly higher in the BJ vs. placebo group (5.4%; $p = 0.034$) and a trend toward significance was observed ($p = 0.055$) in time-to-peak power (−8.4% for BJ vs. placebo). When comparing power variables recorded at the start and end of the test between supplementation groups, average power 0–5 s was significantly higher in BJ (9.5%; p =0.05), while no differences emerged in average power 25–30 s or in the fatigue index ($p = 0.538$).

When we examined average power in 10-s intervals, average power 0–10 s (placebo = 661.44 ± 113.6 W; CV: 17.2%, BJ = 713.03 ± 116.8 W; CV: 16.4%) was higher in BJ (7.8%; $p = 0.022$), but no significant differences between the conditions were produced in average power 10–20 s (placebo = 677.95 ± 110.2 W; CV: 16.3%, BJ = 708.82 ± 121.3 W; CV: 17.1%) or average power 20–30 s ($p = 0.238$ and $p = 0.436$ respectively) (placebo = 502.57 ± 99.6 W; CV: 19.8%, BJ = 523.4 ± 106.8 W; CV: 20.4%) (Figure 1). Figure 2 shows that when considering 15-s intervals, a significant difference between BJ and placebo was produced in average power 0–15 s (6.7%; $p = 0.048$) (placebo = 682.60 ± 108.9 W; CV: 16.0%, BJ = 728.59 ± 118.3 W; CV: 16.2%) but not in average power 15–30 s ($p = 0.365$) (placebo = 545.36 ± 99.9 W; CV: 18.3%, BJ = 568.24 ± 107.9 W; CV: 19.0%).

Figure 3 visually illustrates how the W values recorded were considerably higher during the first seconds of the Wingate test and reached their greatest values in the upper part of the curve while Figure 4 show the individual and mean group response of the main variables analyzed.

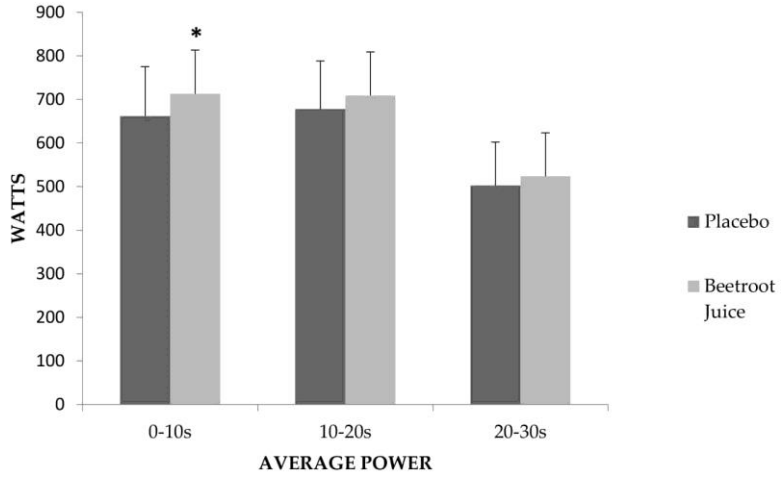

Figure 1. Average power recorded in the intervals 0–10, 10–20 and 20–30 s; * significant difference between beetroot juice and placebo ($p < 0.05$).

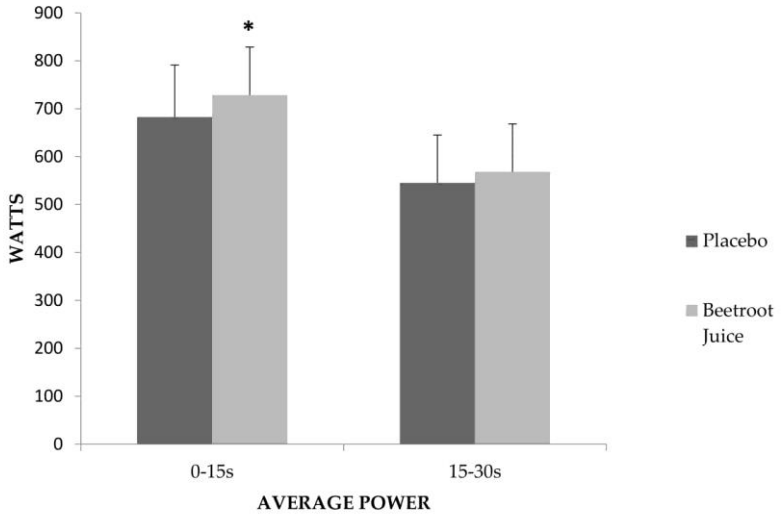

Figure 2. Average power recorded in the intervals 0–15 and 15–30 s; * significant difference between beetroot juice and placebo ($p < 0.05$).

Table 3. Power variables recorded in the Wingate test in participants according to the experimental conditions (beetroot juice or placebo supplementation).

Variables	Placebo	CV (%)	BJ	CV (%)	%	*T*	*p*
Minimun power (W)	433.33 ± 99.39	22.9	442.61 ± 122.79	27.7	2.1	−0.264	0.796
Peak power (W) *	816.83 ± 136.97	16.8	865.69 ± 143.91	16.6	6.0	−2.357	0.034
Mean power (W)	613.98 ± 94.14	15.3	648.41 ± 104.79	16.2	5.6	−1.541	0.146
Time-to-peak power (s)	8.00 ± 1.46	18.3	7.33 ± 1.23	16.8	−8.4	2.092	0.055
Average power 0–5 s (W) *	530.34 ± 106.49	20.1	580.50 ± 109.87	18.9	9.5	−2.141	0.050
Average power 25–30 s (W)	462.46 ± 101.63	22.0	482.28 ± 112.73	23.4	4.3	−0.631	0.538
Fatigue index (%)	46.28 ± 12.01	25.9	48.65 ± 15.54	25.8	5.1	−0.701	0.495

BJ = beetroot juice; s = seconds; W = watts; CV = coefficient of variation; * significant difference for placebo vs. BJ ($p < 0.05$).

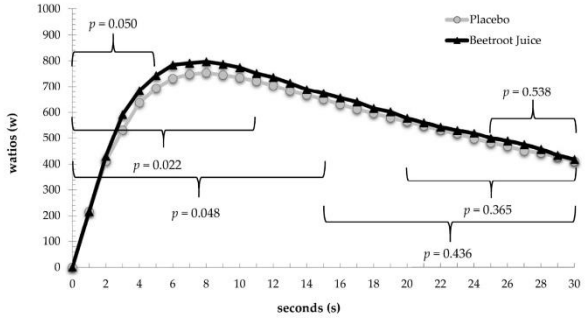

Figure 3. Power curves recorded during the Wingate test in the placebo and beetroot juice supplementation groups. The figure shows that during the first 15 s of the test (0–5 s, 0–10 s and 0–15 s) significant differences in power emerged between the two experimental conditions.

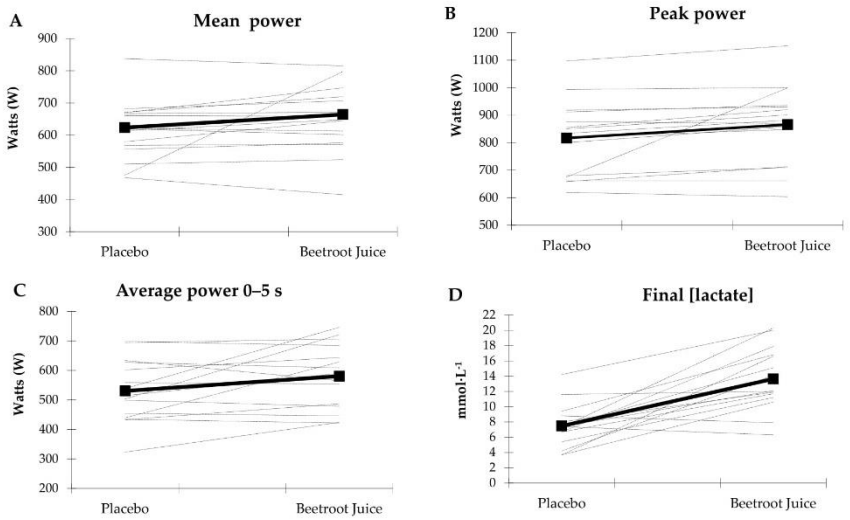

Figure 4. Mean power (**A**); peak power (**B**); average power 0–5 s (**C**) and Final (lactate) (**D**) recorded in all participants (dashed line) and average for the sample (continuous line).

4. Discussion

The main finding of our study was that BJ supplementation was able to significantly improve the power developed during the first 15 s of a Wingate test, with impacts on W_{peak} and a trend towards a shorter time-to-peak power ($p = 0.055$). This improved peak power production produced after the intake of BJ (6%) coincides with the results reported in studies examining its impacts on knee-extension exercises (6%) [41] and on inertial cycle ergometry (6%) [42], though the dose used in our study was 5.6 mmol NO_3^- vs. 11.2 mmol NO_3^- used in other investigations [41,42]. However, in this last study, BJ supplementation led to no such improvements in average and peak power during a Wingate test executed on an isokinetic cycle ergometer rather than an inertial one as in the test employed here [40].

Despite reports of significantly raised NO_2^- levels in response to BJ doses of 8.4 mmol NO_3^- and 16.8 mmol NO_3^- compared with a dose of 4.2 mmol NO_3^- [43], our finding of improved peak power attributable to the intake of 5.6 mmol NO_3^- BJ before the Wingate test supports the results described by Rimer et al. [42] in response to a dose of 11.2 mmol NO_3^-. To explain the lack of an effect of BJ on an isokinetic Wingate test, we need to consider the characteristics of the different ergometers used in the study by Rimer's group. As power reflects the relationship between force and velocity, for an inertial cycle ergometer in which the load remains constant (fixed at a load relative to a percentage of body weight) [44], any changes produced in power production are attributable only to modifications in pedalling cadence [45]. In contrast, when performing the test on an isokinetic cycle ergometer, the pedalling cadence is fixed and power is interpreted as the force exerted at a given velocity. Because pedalling cadence is known to correlate highly with knee and hip angular velocities, this cadence is used to indicate the shortening velocity of the muscles involved in both these joints [45]. Hence, improvements in the power produced when pedalling on inertial cycle ergometers are sensitive to changes in power output associated with velocity, while improvements when using an isokinetic cycle ergometer are related to variations in force. As one of the functions of NO is to reduce muscle shortening velocity [46], the beneficial effects of BJ supplementation may perhaps not be observed when using an isokinetic ergometer.

Among the factors that affect the production of power, we should highlight the influence of the type of muscle motor unit recruited, as type II muscle fibres show a greater contraction velocity and force [47]. Accordingly, it has been observed that the improved peak power produced following BJ intake is specific to type II motor units [48]. Studies in animals have also shown that the effects of BJ supplementation on blood flow [5] and force production [33] are only observed in type II motor units. In effect, studies in animal models have shown that NO increases the effects of acetylcholine exclusively in type II muscle fibres [49]. An improved action of acetylcholine may enhance motor neuron depolarization [49]. Besides, BJ increases the expression of calsequestrin [29], increasing calcium release from the sarcoplasmic reticulum to the muscle fibre sarcoplasm [50]. At this site, calcium binds to tropomyosin and troponin promoting actin and myosin crossover [51]. Increased action potential succession and the presence of calcium could promote trains of action potentials thus increasing peak power output [52]. In effect, this has been observed by monitoring electromyographic activity during maximum intensity efforts [53].

In addition to the effects of BJ supplementation on force production in type II motor units, there have been reports that BJ reduces ATP demands during the exercise effort [4,54], manifesting as the reduced degradation of phosphocreatine (PCr) both in low and high intensity exercise [54]. A diminished PCr cost during the maximum intensity effort would delay the depletion of PCr reserves [6]. Given the essential role of PCr in high intensity efforts [20], its delayed depletion during the Wingate test should help maintain greater power peaks during the first part of the test, thus explaining the significant improvement noted in average power 0–15 s (6.7%).

The effects of BJ reported here are consistent with those of a study in which the effects of supplementation with nitrate salts were examined in a Wingate test, also performed on an inertial cycle ergometer in a population of similar characteristics (CrossFit athletes). Thus, it was observed in CrossFit athletes that supplementation with 8 mmol of potassium nitrate led to a 6.6% improvement in

peak power [55], comparable to the present finding of 6%. Also, while no significant improvement was observed in the average power developed during the test, a trend towards significance was noted ($p = 0.08$) [55]. However, as these authors did not compare power production across test intervals, it is not known whether a significant improvement was produced during the first 15 s of the test as noted in our study.

The effects of BJ [42] or nitrate salts [46] on maximum power produced during cycle ergometry mediated by increased NO concentrations or reduced PCr degradation rate [5,54] could also explain the findings of several studies: a greater number of repetitions (26.1 vs. 21.8) of 15-s bouts of cycle ergometry executed at 170% of maximum aerobic power (MAP) with 30-s rest periods [41]; improved power developed during 24 sets of 6-s work periods and 24-s of rest (~7%) [56]; or improved cycle ergometry work accomplished in 5 sets of 6 s followed by 14 s of rest (~3.5%) in the middle and end of a protocol consisting of 2 × 40 min that simulated the demands of a team sport's match [23].

The increase observed here in blood lactate concentrations (82.6%) in response to BJ supplementation is similar (106.3%) to that detected in rats given an injection of NO_2^- [57]. Blood lactate concentrations are considered to indicate the glycolytic contribution to energy metabolism [54], though the transfer of lactate to the bloodstream depends on the extent of capillarization and muscle perfusion. In a 30-s maximum load test such as the Wingate, in which type II motorneurons are recruited and there is a highly glycolytic metabolism, blood lactate concentrations are much lower than those of muscle lactate and several minutes are needed for blood and muscle concentrations to reach a balance [58]. It is possible that increased blood flow to type II motor units following BJ supplementation [5], could have led to increased blood lactate concentrations [59]. This possible effect of BJ [5] could be responsible for the increase in blood lactate levels observed after the high-intensity effort in our study and in the study by Glean et al. [57].

Another possible explanation for the increase produced in blood lactate concentrations could be elevated glycolytic activity [57]. Accordingly, the increased power produced in our study would be the consequence of a glycolytic type metabolism during the exercise effort. This mechanism could explain the ergogenic effect of this supplement detected in our study and in others [54]. Wylie et al. [56] observed increased blood lactate levels along with improved performance at a cycle ergometer protocol consisting of 24 sets of 6 s and 24-s rest periods, while Mosher et al. [58] noted an increased number of repetitions accomplished until exhaustion when lifting a load equivalent to 60% of one maximum repetition (1 RM) during bench press exercise. As the impacts on power output of BJ supplementation are attributed to the improved performance of type II motor units characterized by a greater dependence on glycolytic energy metabolism, this leads to a greater increase in blood lactate following the exercise effort [59]. In any case, neither of these two explanations are exclusive such that it could be that the increase in blood lactate produced at the end of the test were the consequence of a greater amount of work executed by type II motor units as well as their greater blood supply.

Limitations

Given that the effects of BJ supplementation are mediated by its capacity to raise levels of NO_2^- which later may be reduced to NO, a limitation of our study was that we were unable to measure blood NO_2^- levels. Moreover, since prior studies have shown a greater effect of BJ doses of 8.4 and 16.8 mmol NO_2^- vs. 4.2 mmol NO_2^- to increase blood NO_2^- levels and improve endurance performance [43], we could have compared the impacts of the dose of 5.6 mmol NO_2^- employed with that of a higher dose (11.2 mmol). The increased blood lactate concentrations observed here and in other studies [57] could either be due to a potentiating effect of BJ on blood flow which would accelerate the passage of lactate to the blood or to an increase in glycolytic activity. Hence, by monitoring blood lactate kinetics after the Wingate test, we could have examined whether these higher lactate concentrations persisted during the recovery period (due to increased glycolytic activity) or if the lactate concentration differences would have evened out (indicating increased flow of muscle lactate to the bloodstream as the consequence of an effect on the blood supply to the muscles). A further limitation is that we did

not undertake a test-retest. However, to avoid the possible interaction of the factor time, we randomly assigned the experimental conditions ensuring that 50% of the subjects worked under one or other condition in each test session. In addition, as an inclusion criterion, participants had experience with the Wingate test.

5. Conclusions

Beetroot juice (containing 5.6 mmol NO_3^-) taken as a supplement had an ergogenic effect on maximum power production and a trend was observed for this to occur within the first 15 s of an inertial cycle ergometer Wingate test. The supplement also led to increased blood lactate concentrations post-exercise. We attribute these effects of BJ to specific improvements in power output and blood supply to type II motor units.

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Effects of Beetroot Juice Supplementation on Performance and Fatigue in a 30-s All-Out Sprint Exercise: A Randomized, Double-Blind Cross-Over Study

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Abstract: As a nitric oxide precursor, beetroot juice (BJ) is known to enhance high-intensity exercise performance (80–100% VO_{2max}) yet its impacts on higher intensity sprint exercise (>100% VO_{2max}) remain to be established. This study sought to examine the effects of BJ supplementation on performance and subsequent fatigue during an all-out sprint exercise. Using a randomized cross-over, double-blind, placebo-controlled design, 15 healthy resistance-trained men (22.4 ± 1.6 years) ingested 70 mL of either BJ or placebo. Three hours later, participants undertook a 30-s all-out Wingate test. Before and after the sprint exercise and at 30 s and 180 s post-exercise, three countermovement jumps (CMJ) were performed and blood lactate samples were obtained. Compared to placebo, BJ consumption improved peak (placebo vs. BJ, 848 ± 134 vs. 881 ± 135 W; $p = 0.049$) and mean (641 ± 91 vs. 666 ± 100 W; $p = 0.023$) power output and also reduced the time taken to reach W_{peak} in the Wingate test (8.9 ± 1.4 vs. 7.3 ± 0.9 s; $p = 0.003$). No differences were detected in the fatigue index. In addition, while over time CMJ height and power diminished (ANOVA $p < 0.001$) and blood lactate levels increased (ANOVA $p < 0.001$), no supplementation effect was observed. Our findings indicate that while BJ supplementation improved performance at the 30-s cycling sprint, this improvement was not accompanied by differences in fatigue during or after this type of exercise.

Keywords: nitric oxide; nitrates; muscle power; muscle fatigue

1. Introduction

Dietary nitrate supplementation has been described as a potential ergogenic aid for high-intensity exercise efforts (80–100% VO_{2max}) as it reduces the oxygen cost of ATP synthesis and ATP cost of muscle contraction thus improving muscle contraction/relaxation, force and power production [1–3]. However, the impacts of nitrate supplementation on all-out sprint exercise

performance (>100% VO_{2max}), and particularly its effects on the fatigue induced by this mode of exercise [4–6] have been scarcely addressed.

Ingested nitrate (NO_3^-) is a well-known precursor of nitric oxide (NO) in humans [7]. Around 25% of circulating NO_3^- is taken up by salivary gland acinar cells in a process facilitated by sialin [8,9]. Oral microorganisms, particularly those on the posterior aspect of the tongue, initiate the reduction of NO_3^- into nitrite (NO_2^-), which subsequently in the stomach and gut, can be converted into NO and be absorbed under hypoxic conditions [8–10]. The majority of the remaining NO_3^- and NO_2^- molecules that reach the intestine are absorbed by this organ increasing NO levels in blood [9]. NO offers several exercise adaptation benefits [11] through its effects of inducing vasodilatation, reducing blood viscosity, and promoting muscular oxygen perfusion and gas exchange [12]. In skeletal muscle, NO reduces oxidative stressor production and promotes mitochondrial biogenesis and efficiency [13,14]. Moreover, NO it is also able to increase force and power production during muscle contraction, decreasing the cost of ATP needed as well as the oxygen required to synthesize ATP [1–3].

Beetroot juice (BJ) is a NO_3^--rich supplement commonly used because of its high betacyanin and polyphenol contents that promote NO synthesis to a greater extent than other NO_3^- salts [15,16]. The ergogenic effect of NO_3^- supplementation was initially observed in terms of metabolic adaptations to endurance training [17]. However, despite the known impact of BJ on aerobic performance, recent data indicate a potential effect of NO_3^--rich supplements on anaerobic exercise [4].

Interestingly, the observed benefits of BJ only seem to affect type II muscle fibers [11]. In these fibers, NO stimulates calcium release into the sarcoplasm via calsequestrin upregulation [18] and reduces the phosphocreatine degradation rate, decreasing ATP cost across several ranges of exercise intensity [19]. During sprint exercise (>100% VO_{2max}), type II muscle fibers are mainly recruited to satisfy the high muscle contraction demands. In these glycolytic fibers, exercise leads to a reduced pH in comparison to oxidative fibers. Intra-cell acidity also promotes the reduction of NO_2^- to NO [8]. In turn, the increase in NO availability may diminish the ATP and phosphocreatine required by each muscle contraction with the consequence of an ergogenic effect of NO_3^- supplementation in sprint exercise achieved by improving power production and attenuating the fatigue induced by this exercise mode [20,21].

However, despite acute BJ administration emerging as an effective strategy to improve different modes of exercise performed to exhaustion [22], the influence of this supplement has been scarcely explored in sprint exercise [1–3,20,23,24]. Two studies have shown that BJ supplementation increases peak power output in a 3–4 s [23] or 30 s cycle ergometer exercise [20,23,24]. However, the benefits of BJ on the muscle power produced in a vertical jump have not been investigated. The countermovement jump (CMJ) is a useful test to explore the muscle contractile properties and neuromuscular performance of the lower-limbs [25]. This test has been extensively used in high-intensity sports in which the stretch-shortening cycle plays a pivotal role [26]. Further, given that fatigue can be defined as a reduction in strength or power regardless of the ability to sustain a required task [27], conducting the CMJ before and after an extenuating task is an effective method of monitoring muscle fatigue [28]. In this context, the present study was designed to examine the effects of BJ, as a NO_3^--rich supplement, on performance at a single 30-s all-out sprint exercise and the fatigue caused by the exercise bout. Our working hypothesis was that BJ intake would increase the peak power generated by muscle contraction and reduce the time needed to achieve this peak power output with the consequence of diminished neuromuscular fatigue after the sprint.

2. Materials and Methods

2.1. Participants

Fifteen young men (age 22.4 ± 1.6 years, height 178 ± 6 cm, weight 76.9 ± 10.3 kg) were recruited. All subjects had at least 18 months of experience with resistance exercise, training 3 sessions per week (e.g., bench press and leg press 1RM were 1.0 and 1.5-fold higher than their body mass weight,

respectively) and were familiar with the 30-s all-out Wingate and CMJ tests. Subjects were instructed to refrain from taking sports supplements, medical supplements or any ergogenic aids during the 3 months before the tests and were excluded if they failed to comply. Further exclusion criteria were smoking or cardiovascular, pulmonary, metabolic or neurologic disease.

Candidate participants were first informed of the experimental protocol before giving their written consent. The study was approved by the Ethics Committee of Alfonso X University in (code 1.010.704) accordance with the latest version (7th) of the Declaration of Helsinki.

2.2. Experimental Design

The study design was randomized cross-over, placebo-controlled and double-blind. Participants reported to the laboratory on two separate days under the same experimental conditions (72 h between sessions, 0.5 h difference in test initiation). Participants were instructed to avoid any form of exercise in the 72 h leading up to each test.

In session 1, participants were subjected to a preliminary assessment of body composition and underwent a familiarization session of the experimental protocol. Then, on two separate occasions (sessions 2 and 3) as they arrived at the laboratory, participants were provided with a supplement containing either placebo (placebo) or BJ. The trial was double-blinded such that one researcher (P.V.-H.) allocated all the participants' drinks in a counter-balanced fashion (in each trial 50% of participants ingested placebo and 50% ingested BJ beverages) with random assignment to each supplement (using Excel, Microsoft, Washington, DC, USA) and this researcher did not take part in the subsequent experimental procedures or statistical analysis of data. Three hours after taking the supplement, all participants performed a 30 s all-out Wingate test on a Monark ergometer (Ergomedic 828E, Vansbro, Sweden), as previously described [19]. Strong verbal encouragement was provided in all the sprint tests. In addition, data were collected in three CMJ jumps and blood samples for lactate determination were obtained in duplicate before (Pre) and after the sprint exercise at 30 s (Post) and 180 s post-exercise (Post-3). The study procedure is illustrated in Figure 1.

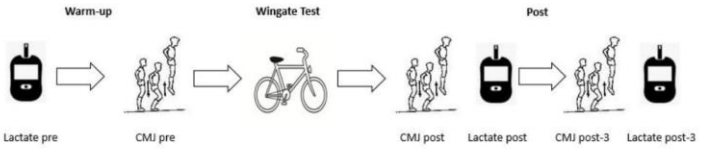

Figure 1. Experimental procedure.

2.3. Placebo vs. BJ Ingestion

After an overnight fast, participants reported to the laboratory 3 h before the first CMJ jump test. Upon arrival, they were provided with either 70 mL of BJ (containing 6.4 mmol of NO_3^-) or the same drink lacking NO_3^- (placebo, 0.04 mmol of NO_3^-) (Beet-It-Pro Elite Shot, James White Drinks Ltd., Ipswich, UK) as described elsewhere [20].

All participants were instructed to follow a diet sheet the day before each trial that consisted of 60% carbohydrates, 30% fat and 10% proteins. Dietary NO_3^- was limited by providing subjects a list of NO_3^--rich foods (e.g., beetroot, celery or spinach) they should avoid in the 48 h before each trial. Also, in the 24 h leading up to each test, subjects were encouraged to avoid brushing their teeth or use an oral antiseptic rinse, or ingest gum, sweets or stimulants (e.g., caffeine) that could alter the oral microbiota and interfere with NO_3^- reduction.

2.4. Sprint Performance Variables

Power output (W) was monitored second-by-second in all sprints. Mean power output (W_{mean}) was calculated as the average power generated during the 30-s test. Peak power output (W_{peak}) was

taken as the highest W value recorded. The time (s) taken to reach W_{peak} was also recorded. Minimum power output (W_{min}) was considered as the lowest W value recorded during the 10 last seconds of the test. Finally, the fatigue index (FI) was calculated using the equation: $FI = (W_{peak} - W_{min})/W_{peak}$. In addition, mean power output in each Wingate test was calculated for the entire test (30 s) and at 10 s ($W_{mean0–10s}$, $W_{mean10–20s}$ and $W_{mean20–30s}$) and 15 s intervals ($W_{mean0–15}$ and $W_{mean15–30s}$) as described elsewhere [19].

2.5. Neuromuscular Fatigue

Neuromuscular fatigue in the legs was measured as the loss of height and power in a CMJ test performed on a force platform (Quattro Jump model 9290AD; Kistler Instruments, Winterthur, Switzerland) [28–30]. Participants were highly familiarized with this vertical jump test. Two CMJ were performed before (Pre) and after the Wingate test at 30 s (Post-1) and 180 s post-exercise (Post-3). At each time-point, mean values of height (cm), mean power (CMJ_{Wmean}) and peak power (CMJ_{Wpeak}) were recorded.

2.6. Blood Lactate

Before the first CMJ and immediately after the subsequent vertical jumps, capillary blood samples (5 µL) were obtained from the index finger of the right-hand for lactate determination using a Lactate ProTM 2 LT-1710 Instrument (Arkray Fatory Inc., KDK Corporation, Shiga, Japan).

2.7. Statistical Analysis

The Shapiro-Wilk test was first performed to assess the distribution of the data. Then paired *t*-tests for normally-distributed data and the Wilcoxon test for non-normally distributed variables (Time-to-W_{peak}, $W_{0–15s}$, $W_{15–30s}$, $W_{10–20s}$ and $W_{20–30s}$) were used to compare all sprint variables between the experimental conditions (placebo vs. BJ). A two-way ANOVA for repeated measures was also used to compare placebo vs. BJ for two between-subject conditions: supplementation (placebo vs. BJ) and time (pre-exercise, 30 s post-exercise and 180 s post-exercise). Before the ANOVA, we confirmed there was no violation of the sphericity assumption using Mauchly's test of sphericity. Holm-Bonferroni was used as post-hoc test when significant differences were detected. Values are provided as the mean ± standard deviation (SD). Significance was set at $p < 0.05$. All statistical tests were performed using the software package SPSS v.18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Sprint Performance Variables

The effects of placebo and BJ on the 30-s all-out sprint test are shown in Table 1. Compared to placebo, BJ supplementation increased W_{peak} (~3.8%; $p = 0.049$) and W_{mean} (~4.0%; $p = 0.023$), while reduced time to W_{peak} (~18%; $p = 0.003$). In 12 of the 15 participants, W_{peak} was higher after BJ administration compared to the placebo condition (Figure 2). In contrast, no significant differences were observed in W_{min} (~4.4%; $p = 0.064$) or FI (~0.22%; $p = 0.914$).

Table 1. Effects of placebo or BJ intake on performance at a 30-s sprint (Wingate) test.

Variable	Placebo	BJ	*p*-Value
W_{peak} (W)	848 ± 134	881 ± 135	0.049
Time to W_{peak} (s)	8.9 ± 1.4	7.3 ± 0.9	0.003
W_{mean} (W)	641 ± 91	666 ± 100	0.023
W_{min} (W)	453 ± 64	472 ± 72	0.064
Fatigue index (FI) (%)	46 ± 8	46 ± 7	0.914

Values are means ± standard deviation. BJ, beetroot juice.

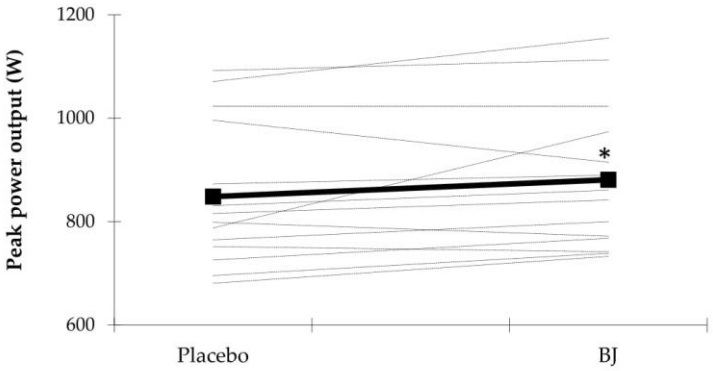

Figure 2. Effects of placebo and BJ intake on W_{peak} after sprint exercise. Means and individual values are shown as a bold or dotted line respectively. * $p < 0.05$ compared to placebo. BJ, beetroot juice.

Values of W_{mean} were recorded in 10 and 15 s intervals. Figure 3 displays W_{mean} values in 15 s intervals ($W_{0–15s}$ and $W_{15–30s}$). An increased W_{mean} was observed after BJ intake compared to placebo during the first 15 s of the sprint (placebo vs. BJ, 709 ± 113 vs. 740 ± 122 $W_{0–15s}$; $p = 0.017$), while no significant differences were recorded during the last 15 s (placebo vs. BJ, 574 ± 80 vs. 593 ± 87 $W_{15–30s}$; $p = 0.173$).

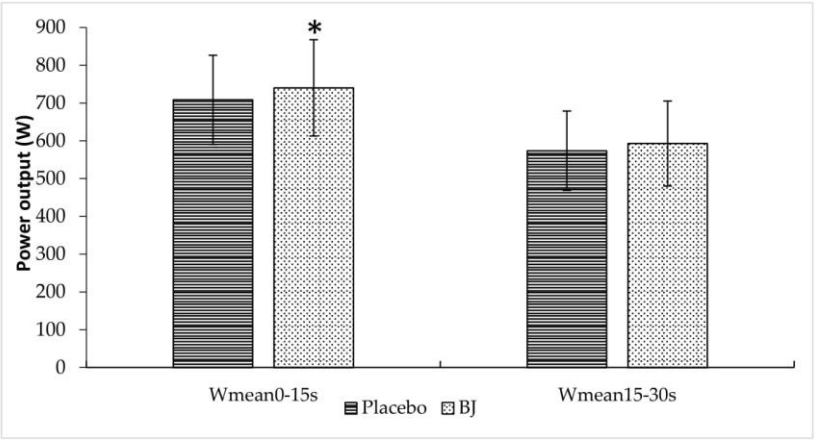

Figure 3. Effects of placebo and BJ intake on W_{mean} values recorded over 15 s intervals (A, $W_{0–15s}$; B, $W_{15–30s}$) after the sprint. * $p < 0.05$ compared to placebo.

Figure 4 provides W_{mean} values in 10 s intervals ($W_{0–10s}$, $W_{10–20s}$ and $W_{20–30s}$). Compared to placebo, a significant increase in W_{mean} was observed after BJ intake during the first 10 s interval (placebo vs. BJ, 683 ± 118 vs. 717 ± 127 $W_{0–10s}$; 5.0%, $p = 0.043$), while no significant differences emerged for the intervals 10–20 s (placebo vs. BJ, 712 ± 105 vs. 735 ± 113 $W_{10–20s}$; 3.2%, $p = 0.078$) or 20–30 s (placebo vs. BJ, 529 ± 73 vs. 548 ± 79 $W_{20–30s}$; 3.6%, $p = 0.30$).

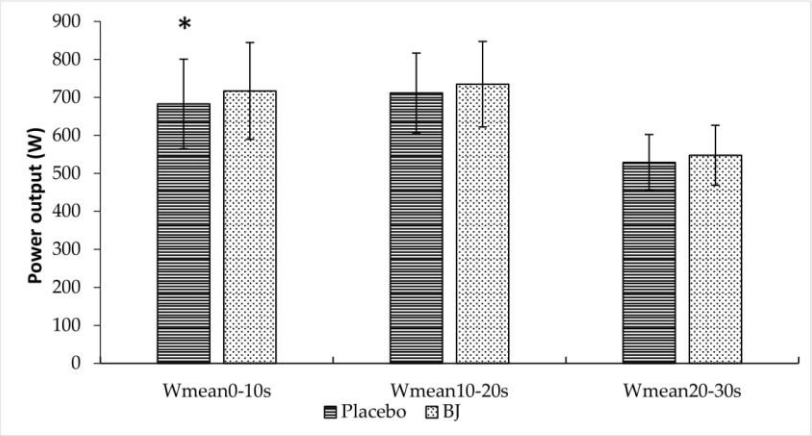

Figure 4. Effects of placebo and BJ intake on W_{mean} values recorded over 10 s intervals ($W_{mean0–10s}$, $W_{mean10–20s}$ and $W_{mean20–30s}$) after the sprint. Values are means ± standard deviation. * $p < 0.05$ compared to placebo.

3.2. Neuromuscular Fatigue and Blood Lactate Concentrations

The effects of placebo and BJ intake on neuromuscular fatigue measured through the CMJ test are shown in Table 2. The 30-s all-out Wingate test led to significant reductions in CMJ_{height}, CMJ_{Wpeak} and CMJ_{Wmean} (ANOVA time effect, $p < 0.001$). Compared to Pre, a significant decrease was observed at Post and Post-3 in CMJ_{height} (Pre vs. Post, ~38%; Pre vs. Post-3, ~19%; $p < 0.001$), CMJ_{Wpeak} (Pre vs. Post, ~28%; Pre vs. Post-3, ~10%; $p < 0.001$) and CMJ_{Wmean} (Pre vs. Post, ~21%; Pre vs. Post-3, ~14%; $p < 0.001$); while a significant increase was observed for Post-3 compared to Post in all variables (~24% CMJ_{height}, ~22% CMJ_{Wpeak}, ~21% CMJ_{Wmean}; $p < 0.001$). No supplementation or interaction effects (supplement × time) were observed.

Figure 5 illustrates the blood lactate values recorded after the sprint test. Blood lactate concentration was significantly higher after the 30-s all-out Wingate test (ANOVA time effect, $p < 0.001$). Compared to Pre (placebo, 1.47 ± 0.71 mmol/L; BJ, 1.47 ± 0.35 mmol/L), blood lactate was significantly higher at the time points Post-0.5 (placebo, 13.86 ± 3.37 mmol/L; BJ, 14.49 ± 3.27 mmol/L; $p < 0.001$) and Post-3.5 (placebo, 15.20 ± 2.62 mmol/L; BJ, 14.84 ± 2.32 mmol/L; $p < 0.001$). No supplementation (ANOVA supplementation effect, $p = 0.858$) or interaction effects (ANOVA supplement × time effect, $p = 0.719$) were detected.

Table 2. Effects of placebo or BJ intake in a neuromuscular fatigue (CMJ) after a 30-s all-out Wingate test.

Variable	Placebo			BJ			Suppl.	Time	Suppl. × Time
	Pre	Post	Post-3	Pre	Post	Post-3			
CMJ_{height} (cm)	30.8 ± 4.6	19.5 ± 5.1 [a]	25.0 ± 4.3 [a,b]	31.5 ± 3.4	19.0 ± 4.2 [a]	25.3 ± 4.2 [a,b]	0.863	<0.001	0.864
CMJ_{Wpeak} (W)	50.5 ± 4.7	36.9 ± 5.9 [a]	45.2 ± 4.6 [a,b]	51.1 ± 3.6	36.6 ± 4.9 [a]	44.7 ± 4.5 [a,b]	0.947	<0.001	0.850
CMJ_{Wmean} (W)	27.3 ± 3.6	20.0 ± 4.2 [a]	24.0 ± 3.8 [a,b]	27.9 ± 3.3	19.6 ± 3.5 [a]	23.8 ± 3.6 [a,b]	0.994	<0.001	0.850

Values are means ± standard deviation. [a] $p < 0.05$ compared to Pre; [b] $p < 0.05$ compared to Post. Pre, before sprint exercise; Post, post-exercise; Post-3, 3 min post-exercise.

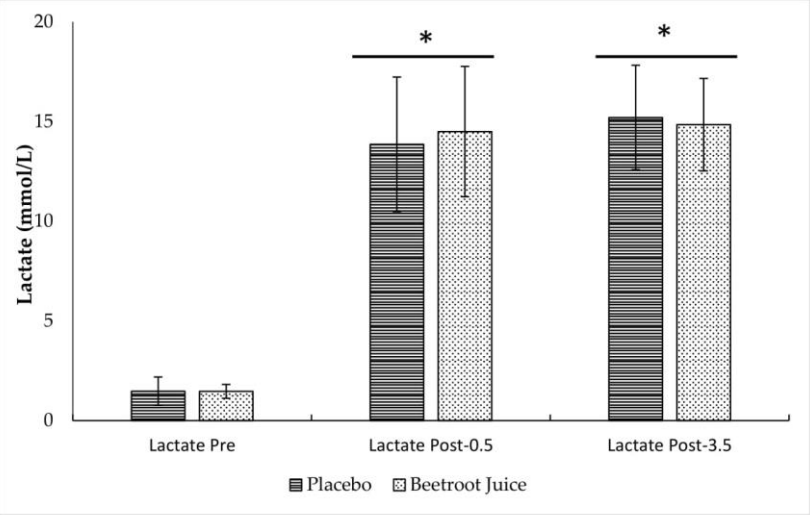

Figure 5. Blood lactate concentrations recorded after the sprint for the placebo and BJ conditions. Values are means ± standard deviation. * $p < 0.05$ compared to Pre. Pre, before sprint exercise; Post-0.5, 0.5 min post-exercise; Post-3.5, 3.5 min post-exercise.

4. Discussion

The findings of our study indicate that BJ supplementation enhances peak and mean power output, particularly during the first half of a 30-s all-out sprint test, reducing the time taken to reach peak power output. Despite this improved sprint performance, neuromuscular fatigue caused by this exercise mode was similar after the intake of BJ or placebo. These observations suggest that NO_3^--rich supplements enhance sprint performance without producing cumulative impacts on fatigue levels.

NO_3^- supplementation has been linked to an increase in W_{peak} generated by leg extension in an isokinetic machine at several angular velocities (from 0 to 6.28 rad/s) in healthy subjects (~5–6%) [2,31] and patients with heart disease (~12%) [32]. There are two reports in the literature of investigations examining the effects of an acute dose of BJ on a 30-s all-out Wingate test [19,23]. In the study by Domínguez et al. [20], a significant increase in W_{peak} was observed (~6%) while Rimer et al. [23] observed no such effect. It should be mentioned that in the study by Rimer's group [23], the 30-s Wingate test was performed after 4 series of 3–4 s all-out sprint trials and 5 min of passive rest; and despite the lack of difference in the Wingate test, the delta change in peak power output produced in the 3–4 sprints indicated an increase of ~6.0% after BJ intake compared to placebo. In the present study, a similar increase in peak power output was observed after the 30-s all-out Wingate test (~4%) and this performance improvement seems to occur during the first 15 s of the sprint and hereafter decline. These data indicate that BJ supplementation may cause a transient ergogenic elevation of peak power output during the first few seconds of sprint exercise, and that this effect could be attenuated after several doses of BJ [6].

The use of an isokinetic or isoinertial cycle ergometer for the sprint test may be a confounding factor when examining the ergogenic effect of BJ supplementation [19,23]. In this study, we used an isoinertial cycle ergometer, which measures power output based on a variable pedaling rate at a fixed load (7.5% body mass) [20]. In contrast, using an isokinetic cycle ergometer, the pedaling rate is predetermined [23]. Pedaling rate is strongly related to the angular velocity of the knee and hip, and

can be used as an indicator of muscle contraction velocity [33] and type II motor unit recruitment [34]. An ergogenic effect of BJ intake has been observed not only in sprint exercise [20] but also in other tasks (e.g., leg extension) under elevated angular velocities [2,31,32]. Consistent with this idea, the present data revealed a greater effect of BJ on sprint performance (W_{peak} and time to W_{peak}) for the higher angular velocities.

Animal studies have shown that NO increases acetylcholine activity, particularly in type II motor units, which amplify depolarization of the muscle fibers [35] whereas BJ supplementation induces the elevation of intracellular Ca^{2+} concentrations accompanied by calsequestrin 1 and dihydropyridine receptor upregulation in fast-twitch muscles [18]. Although these mechanisms have not yet been proven in humans, NO_3^- supplementation likely increases force production by inducing type II muscle fiber depolarization and increasing myoplasm Ca^{2+} concentrations facilitating muscle contraction [18,36] by increasing the number of actin-myosin cross-bridges [37]. This improvement in muscle force production in response to BJ consumption has been detected as a higher rate of force development (RFD) [37] through increased peak power output, the time taken to reach that power output and a faster reaction time [4]. In effect, Time to W_{peak} and reaction time are key factors in sports performance, particularly in disciplines in which acceleration determines performance [38,39]. Here, BJ supplementation led to a pronounced reduction in Time to W_{peak} during a 30-s all-out Wingate test, coinciding with previous data in which the increase in W_{peak} was accompanied by a shorter time needed to reach W_{peak} [20]. A reduced Time to W_{peak} was also found when a transient increase in W_{peak} was not detected after prolonged doses of BJ supplements and repeated sprint exercise [6]. In these two previous studies [6,20], the shortened Time to W_{peak} was lower (~0.7 and ~0.2 s, respectively) than the difference observed here (~1.6 s). The greater improvement in Time to W_{peak} reported here may be explained by a reduced level of anaerobic training of our subjects compared to participants of the studies by Dominguez et al. [20] and Jonvik et al. [6], who were well-trained in anaerobic disciplines.

Anaerobic pathways supply ~75% of energy requirements in a 30-s all-out sprint exercise [40,41]. During the first 6 s, ready to use sources of energy are needed to produce maximal peak power output in the shortest time possible. Accordingly, free ATP and PCr stores are critical during the initial part of a sprint [42]. At this time (first 5–10 s), a marked depletion in PCr stores occurs and this compromises power output coinciding with the time at which glycolysis attains its maximum rates [43]. Along with an increased force production capacity, BJ supplementation leads to the reduced ATP cost of muscle contraction [19,44] perhaps by reducing PCr degradation rates. The reduced ATP requirements of muscle contraction together with the maintenance of free ATP and PCr stores promoted by NO_3^- supplementation may give rise to a higher power output during a longer period of time coinciding with the increase in mean power output produced during the first 15 s of the sprint after BJ intake.

Since BJ consumption led to elevated peak and mean power output during the first 15 s of the sprint, we could argue that the muscular fatigue that takes place during the last 15 s and at the end of the sprint will be exaggerated.

The fatigue index calculated during the sprint indicated no differences between the supplements. In addition to the mentioned maintenance of anaerobic sources of energy production, the contribution of aerobic energy production increases during the last 15 s of a Wingate test [41,43]. Since NO_3^- supplementation is known to reduce the oxygen cost of ATP synthesis [45] and to preserve ATP and PCr stores [19], the lack of differences between supplements (placebo vs. BJ) may be explained by a higher capacity of NO_3^- to induce ATP store maintenance and thus reduce the cost of its synthesis by both aerobic and anaerobic sources.

Immediately after the sprint exercise, two CMJ jumps were performed at 30 s and 180 s. CMJ is a vertical jump test that assesses muscle contractile properties and neuromuscular performance (anaerobic power) of the lower-limbs [46,47]. Variables such as CMJ height and power have also been used as indicators of neuromuscular fatigue [48,49]. Some authors have argued that the CMJ test after extenuating exercise [28] serves to assess muscle capacity to replenish ~50% of depleted PCr stores at 30-s post-exercise [50] and to recover almost completely depleted PCr stores at 180 s post-exercise [51].

Hence a pronounced reduction in CMJ performance (height and power) after 180 s will reflect the diminished PCr store replenishment capacity of muscle fibers affecting the stretch-shortening cycle and force production [52]. The present observations are in good agreement with prior findings in which an effect of time in reducing CMJ height and mean power output was seen after a 30-s all-out Wingate test [28–30]. The decrease in CMJ performance was more pronounced at 30 s (~30%) compared to 180 s post-exercise (~10%). However, no differences between supplementation conditions were observed.

In our study, BJ supplementation overall did not give rise to a greater fatigue index during the second half of the test or to neuromuscular fatigue as measured in CMJ tests, after the 30-s all-out sprint test. These results indicate that the improved sprint performance induced by BJ as a NO_3^--rich supplement may not be accompanied by more fatigue.

5. Limitations

Our study has several limitations. BJ is a NO_3^--rich supplement known to increase circulating NO_2^- and NO levels [7]. However, these levels were not measured before the intake by the participants of placebo or BJ. Further, the number of subjects recruited (N = 15), although appropriate for this type of study, limits the detection of small changes that could be the consequence of BJ administration. Finally, participants were not trained cyclists and therefore the ergogenic effects produced by BJ cannot be directly transferred to this sports modality. On the up-side, however, the inclusion of resistance trained individuals was useful to explore the physiological effects of BJ supplementation on skeletal muscle power production and to examine fatigue induced by a sprint exercise to exhaustion.

6. Conclusions

In conclusion, BJ supplementation produced an ergogenic effect in a 30-s all-out Wingate test in terms of increasing W_{peak}, Time to W_{peak} and W_{mean}, particularly during the first half of the sprint, without increasing muscular fatigue accumulation during or after this extenuating sprint exercise. These findings suggest that NO_3^--rich supplements could be a suitable strategy to improve performance in sports modalities in which power and acceleration largely determine performance.

Author Contributions: R.D. and S.F.d.S. designed the experiment; P.J. and P.V.-H. recruited the subjects and hosted the informative session; P.V.-H. and L.G.G.-R. checked that subjects followed the diet guidelines and the timing of supplement ingestion. R.D., E.C., P.J., P.V.-H. and L.G.G.-R. performed the experiments; E.C., A.P.-L. and S.F.d.S. analyzed the data; R.D. and S.F.d.S. conducted the statistical analysis; and R.D., E.C., P.J., P.V.-H., L.G.G.-R. A.P.-L. and S.F.d.S. wrote the manuscript.

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