INTERNATIONAL DOCTORAL THESIS / TESIS DOCTORAL INTERNACIONAL

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Supervised by Paulino Padial Puche PhD

Effect of hypertrophy training at moderate altitude on the response of metabolic stress markers and associated muscle growth mechanisms.

Efecto del entrenamiento de hipertrofia en altitud moderada sobre la respuesta de marcadores de estrés metabólico y mecanismos de crecimiento muscular asociados.

Doctoral Programme in Biomedicine
Programa de Doctorado en Biomedicina

University of Granada
Universidad de Granada

September 2023

UNIVERSIDAD DE GRANADA

Editor: Universidad de Granada. Tesis Doctorales
Autor: Cristina Benavente Bardera
ISBN: 978-84-1195-039-8
URI: https://hdl.handle.net/10481/84700

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Editor: University of Granada. Doctoral thesis.
Cover design & Layout: Eugenia Porcel Rubio.
Proof reading: Eva María Domínguez Jiménez.

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» Acknowledgements

Antes de todo, la realización de esta tesis doctoral y todo el proceso que conlleva ha sido fruto del trabajo, ayuda y apoyo incondicional de todas aquellas personas sin las cuáles no habría sido posible. Y es que este trabajo no se hace solo, sino que es necesario un equipo en el que cada uno aporte su granito de arena.

Por ello, en primer lugar, quiero agradecer la realización de este trabajo al jefe de este equipo y director de la tesis, Paulino Padial. Has sido y serás mi figura de referencia, el "coach", por toda la ayuda, ánimos y tranquilidad que nos aportas. He de decir que de mayor me gustaría ser como tú ya que he aprendido muchísimo de tu forma de ser, de tu forma de impartir clase y sobre todo, de tu forma de entrenar. Por otro lado, agradecer todo el trabajo a la verdadera jefa de este equipo, Belén Feriche. Aunque hemos pasado buenos, y no tan buenos momentos, sé que quieres lo mejor para mí y para mi futuro. Admiro tu forma de trabajar, la dedicación y ganas que le pones y tu forma de transmitir tu trabajo con pasión (aunque a veces no hace falta que transmitas esa pasión los domingos por la tarde).

Y dentro de este equipo, no puede faltar el agradecimiento a mis dos senseis. Por un lado, agradecer a Juan Bonitch el que un día decidiera cogerme como alumna de TFG e iniciarme en este mundillo de la investigación (y hacerme ver que el verdadero deporte es el judo). Y por otro lado, a Filipa Almeida, por enseñarme lo que significa la perfección, estar ahí en todo momento y ofrecerme lo mejor de ti cada día (y por traducir los textos) ¡Gracias senseis! También a todos los que habéis pasado por el laboratorio en estos últimos años para aportar vuestro granito de arena: Irene, Sergio, Lara e Iñaki. Tampoco podría faltar toda la gente del CAR de Sierra Nevada, en especial a Javi, Blanca y Carmen. Gracias por acogernos cada vez que vamos. Y por supuesto, al otro laboratorio, a Pepi. Muchas gracias por dejarme entrar, depositar toda tu confianza en mí y enseñarme todas esas técnicas y experimentos con tanto cariño (aunque creo que debería hacer una carrera para entenderlo algún día).

Gracias a mi otro equipo, el del gimnasio. Es muy importante tener un sitio y personas que te ayuden a desconectar, con las que tomarse un refresco, reírse

de las “personas con movilidad reducida” (entre ellas yo), establecer conversaciones políticas muy profundas y con las que conversar de la tesis mientras se hace el WOD. Gracias también al gran sensei, Juan, al que le prometo volver a hacer judo aunque me de unas cuantas piñas cada vez que entro al tatami. A mis compis de judo, María, Paula, Marta y mi uke favorita, Tere, con las que habéis compartido mis penas y alegrías.

Tampoco puedo olvidarme de Gonzalo, por abrirme las puertas de su laboratorio en mi corta y “lluviosa” estancia en Coruña y por desplazarse hasta Granada con esos aparatos que parecen de la NASA para transmitirnos su pasión por lo que le gusta. Y también a Rafa y Guillermo, por acogerme en su laboratorio durante mi estancia en Cáceres y enseñarme a llevar un proyecto desde todos los puntos de vista.

I would like to thank Brad Schoenfeld and the Lehman College research team (Max, Ryan, Francesca, Alec, Kae, Ji, Jaime, DJ) for allowing me to work with all of you. I have learn a lot about the way you carry out all the experiments and train the people. Thank you for your patient trying to understand me and letting me speak with Brad in Spanish.

Agradecer también la ayuda de todos aquellos voluntarios que han participado en alguno de los experimentos. Perdón por los litros de sangre que os he robado y gracias por aguantar con una sonrisa condiciones extremas como entrenar en una tienda de hipoxia al 99% de humedad.

A todas mis amigas de la carrera con las que he pasado unos 6 años compartiendo emociones, clases al sol a las 2 de la tarde, lentejas antes de balonmano, trabajos grupales, apuntes de clase y exámenes. En especial a Patri, por preocuparse por mí y yo por ella. Porque de vez en cuando me llamas para preguntarme como estoy y actualizar nuestras vidas (aunque se que lo haces porque te aburres en el coche). Caro, por todos esos momentos compartidos y por darle sentido y cordura a lo que hacíamos. Nuria, por tu personalidad tan especial, y por darle vidilla a las situaciones. Isa, mi gran descubrimiento y con la que me iría de viaje a todos sitios. Y Lucía, que aunque no habláramos mucho, creo que eres de esas personas

que actúan en la sombra para que el grupo esté más unido. Se que os irá genial allá donde estéis aunque me gustaría compartir más momentos con vosotras.

A mis amigos/familia de pega, por ayudarme también en este proceso y de los que me alegro haber conocido y que hayan entrado en mi vida. Eva, Eu y Jesús. A parte del agradecimiento personal, señalar a Eva Domínguez, por revisar la tesis y hacer que el inglés esté bien escrito. Espero que hayas aprendido algo leyendo cosas raras. Y a Eugenia Porcel por la gran profesionalidad y paciencia con la que ha maquetado esta tesis.

Por último, y no menos importante, a mi familia. A mis padres, por vuestro apoyo desde el principio (aunque me digáis todos los días que cuándo voy a dejar de estudiar). Gracias por entenderme, aguantar mis barraqueras y esas interminables horas hablando (aclaración: hay que pertenecer a este rango para escucharme hablar). A mi hermano favorito, que siempre está ahí para apoyarme y darme consejo. A mi tita Maru, por cuidarme como si fuera su hija y todo lo que eso conlleva. Y a mis abuelos, que aunque no estén ya aquí, seguro que están orgullos de mí (aunque no entendieran que era eso de la gimnasia).

A Fran, mi apoyo, mi prometido (aunque no me ponga el anillo), el amor de mi vida. Espero que no me manden muy lejos aunque ya buscaremos la forma de estar juntos. Gracias por aguantar lo cabezona que soy y apoyarme en todo momento, por tocarme canciones al piano aunque no sepas, por ser como eres y calmarme cuando estoy triste.

"El ayer es historia, el mañana es un misterio,
el hoy es un regalo, por eso se llama presente"

—

Maestro Oogway

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Abbreviations

Abbreviation	Term
1RM	1-repetition maximum
AKT	Protein kinase B
AMS	Acute mountain sickness
BFR	Blood flow restriction
BP	Barometric pressure
Ca^{2+}	Calcium
CHT	Continuous hypoxic training
CSA	Muscle cross-sectional area
eIF2B	Eukaryotic initiation 2B
Eq FiO_2	Equivalent of the FiO_2
Eq PIO_2	Equivalent of the PO_2
FiO_2	Inspired fraction of oxygen
FOXO	Forkhead box O
GH	Growth hormone
GSK3	Glycogen synthase kinase-3
H^+	Hydrogen ion
HH	Hypobaric hypoxia
HPA	Hypothalamic-pituitary-adrenal
IHE	Intermittent hypoxic exposure
IHT	Intermittent hypoxic interval training
IGF-1	Insulin-like growth factor 1
IL	Interleukin
LHTH	Live high-train-high
LHTHL	Live high-train high and low

Abbreviation	Term
LHTL	Live high-train low
LHTLH	Live high-train low and high
LLTH	Live low-train high
NFATs	Nuclear factor of activated T-cells
NH	Normobaric hypoxia
MAPK	Mitogen-activated protein kinase
MGF	Mechano-growth factor
MiRNA	MicroRNA
MuRF1	Muscle ring finger-1
MyoD	Myoblast determination protein 1
mTOR	Mammalian target of rapamycin
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PO_2	Partial pressure of oxygen
PPAR	Peroxisome proliferator-activated receptor
Pi	Inorganic phosphate
PI3k	Phosphatidylinositol 3-kinase
ROS	Reactive oxygen species
RPE	Rate of perceived exertion
RSH	Repeated sprint training in hypoxia
R_T	Resistance training
RTH	Resistance training under hypoxic conditions
RTN	Resistance training under normoxic condition
SpO_2	Arterial oxygen saturation levels
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α

Abstract

Resistance training (R_T) is a well-established interventional strategy for increasing muscle strength and hypertrophy. However, the available systematic reviews display discrepancies among training methodologies used which makes it difficult to draw firm conclusions about the potential benefit of R_T in hypoxia (RTH) compared to the equivalent training in normoxia (RTN). Seven separate research experiments were carried out to 1) determine the current status of knowledge of RTH on muscle hypertrophy (Section I); and to 2) analyze the effect of the R_T exercise under the two types of acute (Section II and III) and chronic (Section IV) moderate hypoxia conditions (terrestrial vs. simulated) on muscle mass and strength performance markers.

The first study, a systematic review and meta-analysis on the topic, found similar improvements in muscle cross-sectional area (CSA) (SMD [CIs]=0.17 [-0.07; 0.42]) and 1RM (SMD=0.13 [0.0; 0.27]) between RTH and RTN. However, sub-analyses indicated that hypertrophy appears to benefit from shorter (≤60s) inter-set rest intervals during RTH while greater gains in strength were achieved with longer rest intervals (≥120s). Moderate loads (60-80% 1RM) enhanced both hypertrophy and strength. The use of moderate hypoxia (14.3-16% FiO_2) seemed to somewhat benefit hypertrophy but not strength.

The two preliminary studies (study 2 and 3) examined the effects of a hypertrophic R_T session at acute terrestrial hypoxia on serum biomarkers associated with muscular adaptations. In a counterbalanced fashion, 13 resistance trained males completed a R_T session (6 exercises x 3 sets x 10 RM; 120 s min rest) at both moderate altitude (HH; 2320m asl) and normoxia conditions (N; <700m asl). Venous blood samples were taken before and throughout the 30 min post-exercise period for determination of metabolites (lactate), ions (inorganic phosphate [Pi], liquid carbon dioxide [CO_2L], calcium [Ca^{2+}]), cytokines (IL-6, IL-10, TNFα), hormones (growth hormone [GH], cortisol [C], testosterone [T]) and miR-378. Session-related performance and perception of effort (RPE-30) were also monitored. The results showed no

differences in performance and RPE-30. All blood variables displayed statistically significant changes compared to basal levels ($p<0.05$), while miR-378, T and inflammatory responses remained near pre-exercise conditions. No altitude effect was observed in maximal blood lactate, Ca^{2+} and anabolic hormones ($p>0.05$), although the CO_2L reduction in HH ($p<0.001$) seems compatible with an increase in buffering capacity. At HH, the R_T session produced a moderate to large increase in the absolute peak values of the studied cytokines. miR-378 revealed a moderate association with GH and C in both N and HH ($r>0.051$; $p<0.05$).

The studies 4, 5 and 6 aimed to analyze the combined effect of the type of acute hypoxia (terrestrial vs. simulated) and the inter-set rest configuration (60 vs. 120 s) during a hypertrophic R_T session on physiological, perceptual, muscle performance and serum markers. Sixteen active men were randomized into two groups (HH: 2320 m asl; vs. normobaric hypoxia, NH: FiO_2 = 15.9%) and completed four R_T sessions: two under normoxia and two under the corresponding hypoxia condition at each prescribed inter-set rest period. Volume-load, muscle oxygenation (SmO_2) of the vastus lateralis and heart rate (HR) were monitored during training and RPE-30 was determined at the end of the recovery period. Maximal blood lactate [maxLac], circulating hormones, ions (Ca^{2+}, Pi, and CO_2L), cytokines (irisin and myostatin) and miRNAs (miR-378, miR-206 and miR-29c) were measured throughout the initial 30 min post exercise. Volume-load was similar in all environmental conditions and inter-set rest periods. Shorter inter-set rest periods displayed greater increases in maxLac, HR, RPE-30, CO_2L, Pi, C and GH in all conditions ($p<0.05$). Compared to HH, NH showed a moderate reduction in the inter-set rest-HR ($ES>0.80$), maxLac ($ES>1.01$) and SmO_2 ($ES>0.79$) at both rest intervals. Additionally, higher values of circulating Ca^{2+} and Pi, and lower CO_2L, were observed after training in HH compared to NH. The exercise with 60 s rest revealed a large early decrement of irisin in HH with respect to N and NH ($ES < -1.10$; $p = 0.048$). Both hypoxias moderately reduced circulating myostatin after exercise by a similar proportion ($ES < 0.23$; $p > 0.21$). Moderate

to large significant increments in miR-378 and miR-29c were detected in N, HH and NH. Compared to HH, a moderate to large rise in miR-29c and miR-206 was found in NH (ES > 0.96; $p < 0.08$).

The study 7 aimed to analyze the effect of a R_T period at terrestrial and simulated hypoxia on both muscle hypertrophy and maximal strength development with respect to the same training in normoxia. Thirty-three strength-trained males were randomly assigned to N (FiO_2 = 20.9%), HH (2,320 m asl.) or NH (FiO_2 = 15.9%). Subjects completed an 8-week R_T program comprised by 3 sessions/week (full body routine of 6 exercises; 3 sets x 6-12 repetitions, 65-80% 1RM and 90 s rest). Muscle thickness of the lower limbs and 1RM in back squat (1RMSQ) and bench press were assessed on weeks 1, 6 and 8 of the training program. Maximal blood lactate, circulating cytokines (IL-6, IL-10, TNFα), hormones (GH, IGF-1), % active mTOR and miRNAs (miR-206, miR-378 and miR-29c) were measured before and throughout the initial 30 min post-R_T exercise after the first (S1) and last (S22) session. R_T program increased 1RM in all groups ($p>0.001$). NH reached a large significant enhancement compared to N in 1RMSQ (ES=1.20). Muscle growth similarly improved in N and HH after the R_T program (ES= -0.14; p=1.0), while NH remained near to the pre-training values (ES= 0.23; p= 0.160). Similar blood lactate increments were found after S1 and S22 in all groups (p=0.895; η^2_p=0.001). Compared to N, HH and NH groups increased IL-6 and TNFα in S1 (ES >1.12; $p<0.022$), returning near to resting values at the end of the training period. Post-exercise GH increased in all conditions, although no changes were detected in the serum IGF-1. HH group showed a moderate to large increment in % active mTOR after S1 with respect to N (ES=1.04; p=0.017) and NH (ES=1.34; p=0.002) with no differences among groups after S22. The NH group displayed the highest serum miR-206 and miR-29c values ($p<0.020$). Moderate to large nonsignificant increases in serum miR-206 above pre-training values (ES>0.76) and a slight reduction below resting values of miR-29c were depicted in N and HH groups ($p<0.08$).

Results under acute hypoxia suggest that the acute metabolic and physiological responses of a hypertrophic RT exercise are mediated by rest intervals between sets and the type of hypoxia. Altered circulating ions, myokines and miRNA could indicate acute differences in the type of hypoxia on muscle signalling pathway activation after a RT. Overexpression of miR-206 in acute NH could indicate a muscle preservation tendency and interfere with muscle growth after longer training periods. However, the results obtained in this study do not support the expected added benefit of RTH compared to RTN on muscle mass development, although it seems to favour gains in strength. The greater muscle growth achieved in HH over NH confirms the impact of the type of hypoxia on the outcomes. This is supported by the acute and chronic response of some of the evaluated biomarkers. Future research should elucidate the impact of RT and the role of hypoxia on serum biomarkers associated with muscle growth and the adaptation of other non-structure factors related to muscle strength development.

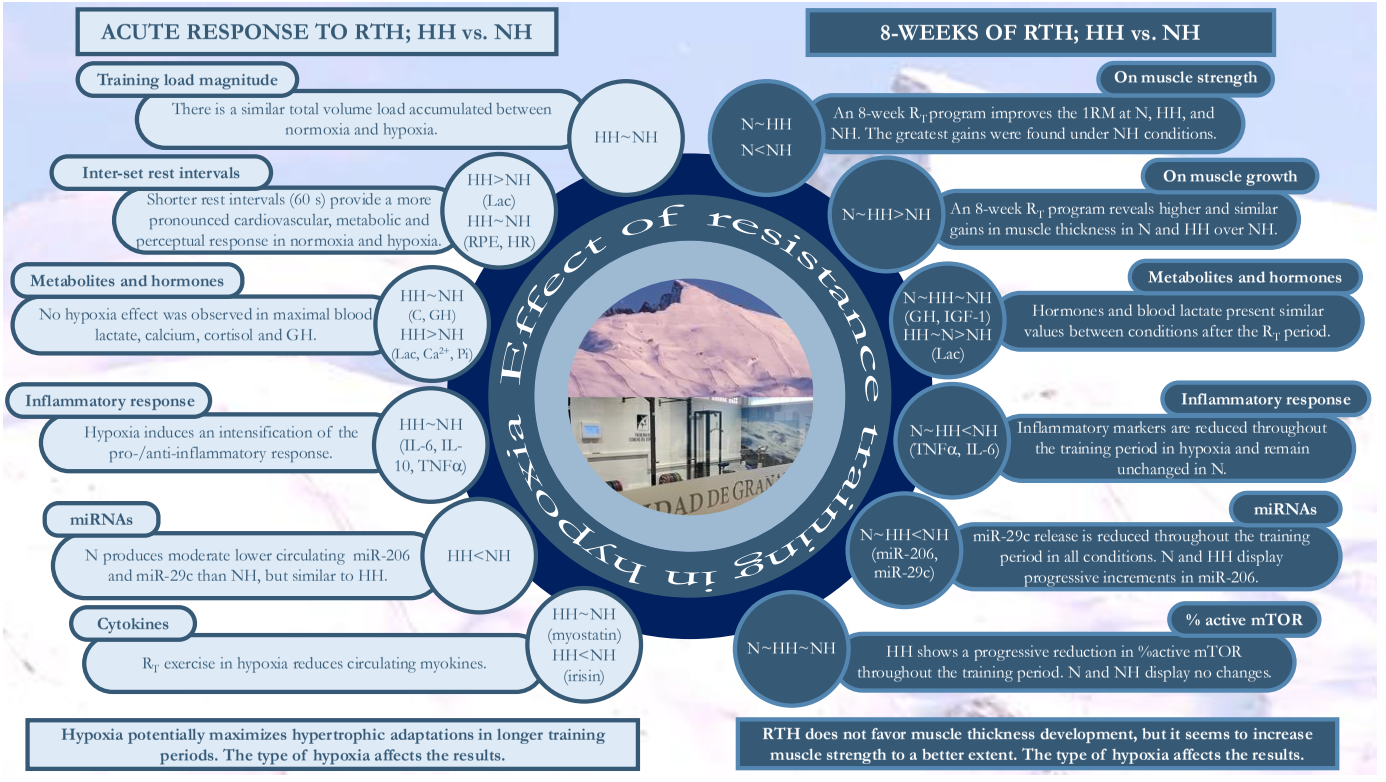


Figure 1 — Graphical abstract. RTH: resistance training in hypoxia; RTN: resistance training in normoxia; HH: hypobaric hypoxia; NH: normobaric hypoxia; N: normoxia; IL: interleukin; TNFα: tumor necrosis factor α; C: cortisol; GH: growth hormone; Lac: lactate; Ca^{2+}: calcium; Pi: inorganic phosphate; RPE: rate of perceived exertion; HR: heart rate; miR: microRNA; R_T: resistance training; 1RM: one repetition maximum; IGF-1: insulin-like growth factor 1; mTOR: mammalian target of rapamycin; % active mTOR: = phospo mTOR/ total mTOR x 100.

Resumen

El entrenamiento de fuerza (R_T) en hipoxia (RTH) se presenta como una estrategia potencialmente beneficiosa para el aumento de la fuerza muscular y la hipertrofia. Sin embargo, las últimas revisiones sistemáticas muestran discrepancias entre los procedimientos utilizados entre los estudios, lo que dificulta establecer conclusiones firmes sobre su beneficio con respecto al mismo entrenamiento en normoxia (RTN). Hemos llevado a cabo siete estudios para 1) determinar el estado actual del conocimiento del RTH sobre la hipertrofia muscular (Sección I); y 2) analizar el efecto del R_T en dos tipos de hipoxia moderada (terrestre vs. simulada) aguda (Sección II y III) y crónica (Sección IV) sobre la hipertrofia y marcadores asociados y el desarrollo de la fuerza máxima.

El primer estudio, una revisión sistemática y metaanálisis, estableció mejoras similares en el área de sección transversal (CSA) (SMD [IC] = 0,17 [-0,07; 0,42]) y la fuerza máxima (1RM) (SMD = 0,13 [0,0; 0,27]) entre RTH y RTN. Sin embargo, los resultados del subanálisis complementario sobre el impacto de la duración de los intervalos de descanso, la intensidad de la carga y la severidad de la hipoxia indicaron que en hipoxia 1) la hipertrofia se beneficia más con la utilización de descansos cortos entre series (≤60 s), mientras que la mayor ganancia de fuerza lo hace con descansos largos (≥120 s); 2) las cargas moderadas (60-80% 1RM) mejoran tanto la hipertrofia como la fuerza en hipoxia; 3) la hipoxia moderada (14,3-16% FiO_2) parece mejorar la hipertrofia en mayor medida, mientras que la ganancia de fuerza se muestra independiente de la severidad de la hipoxia.

Los dos estudios preliminares (estudio 2 y 3) examinaron los efectos de una sesión de R_T orientada a la hipertrofia sobre biomarcadores séricos asociados a las adaptaciones musculares en hipoxia terrestre. De manera aleatoria, 13 hombres con experiencia en el entrenamiento de fuerza ejecutaron una sesión de R_T (6 ejercicios x 3 series x 10 RM; 120 s min de descanso) en altitud moderada (HH; 2320 m s.n.m.) y en normoxia (N; <700 m s.n.m.). Antes y durante los 30 min de

recuperación posteriores al ejercicio se tomaron muestras de sangre para la determinación de metabolitos (lactato), iones (fosfato inorgánico [Pi], dióxido de carbono líquido [CO_2L], calcio [Ca^{2+}]), citoquinas (IL-6, IL-10, TNFα), hormonas (hormona de crecimiento [GH], cortisol [C], testosterona [T]) y miR-378 en suero. También se controlaron variables de rendimiento y la percepción de esfuerzo (RPE-30). Los resultados no mostraron diferencias en el rendimiento y la RPE-30. La respuesta de miR-378, T e inflamatoria se mantuvieron cercanas a condiciones previas al ejercicio. No se observó un efecto de la altitud en el lactato máximo, Ca^{2+} y hormonas anabólicas ($p>0,05$), aunque la reducción de CO_2L en HH ($p<0.001$) parece relacionarse con una mejora de la capacidad "buffer". En HH, la sesión de R_T produjo un aumento de moderado a grande en los valores máximos de las citoquinas estudiadas. El miR-378 reveló una asociación moderada con GH y C tanto en N como en HH ($r> 0.051$; $p <0.05$).

Los estudios 4, 5 y 6 tenían como objetivo analizar el efecto combinado del tipo de hipoxia aguda (terrestre vs. simulada) y el tiempo de descanso entre series (60 vs. 120 s) durante una sesión de R_T sobre el rendimiento fisiológico, perceptivo y muscular y marcadores en suero. Dieciséis hombres activos fueron distribuidos de manera aleatoria en dos grupos (HH: 2320 m s.n.m.; vs. hipoxia normobárica, NH: FiO_2 = 15,9 %) y completaron cuatro sesiones de R_T: dos en normoxia y dos en la condición de hipoxia correspondiente con 60 y 120 s de descanso. El volumen de entrenamiento, la oxigenación muscular (SmO_2) del vasto lateral y la frecuencia cardíaca (FC) se monitorearon durante el entrenamiento y se determinó el RPE-30 al final del período de recuperación. Se midió el lactato sanguíneo para la determinación de su valor máximo [maxLac], hormonas (C y GH), iones (Ca^{2+}, Pi y CO_2L), citocinas (irisina y miostatina) y los mi-ARNs (miR-378, miR-206 y miR-29c) durante los 30 minutos posteriores al ejercicio. El volumen de entrenamiento fue similar en todas las condiciones ambientales y períodos de descanso entre series. Descansos más cortos entre series mostraron mayores aumentos en maxLac, FC, RPE-30, CO_2L, Pi, C y

GH en todas las condiciones ($p<0.05$). En comparación con HH, el grupo de NH mostró una reducción moderada de la FC de descanso entre series (ES>0.80), el maxLac (ES>1.01) y la SmO_2 (ES>0.79) en ambos periodos de descanso. Además, se observaron niveles más elevados de Ca^{2+} y Pi, y niveles más bajos de CO_2L después de la sesión de entrenamiento en HH en comparación con NH. El ejercicio con 60 s de descanso entre series reveló una disminución temprana de irisina en HH con respecto a N y NH (ES < −1,10; p = 0,048). Ambos tipos de hipoxia redujeron moderadamente los niveles de miostatina después del ejercicio en una proporción similar (ES < 0,23; p > 0,21). Se detectaron incrementos significativos de moderados a grandes en el miR-378 y el miR-29c en N, HH y NH. En comparación con HH, la condición de NH registró un aumento de moderado a grande en los niveles circulantes de miR-29c y miR-206 (ES > 0,96; p < 0,08).

El estudio 7 tuvo como objetivo analizar el efecto de un período de R_T en hipoxia terrestre y simulada sobre la hipertrofia muscular y el desarrollo de fuerza máxima con respecto al mismo entrenamiento en normoxia. Treinta y tres hombres con experiencia en el entrenamiento de fuerza fueron distribuidos aleatoriamente en N (FiO_2 = 20,9 %), HH (2320 m s.n.m.) o NH (FiO_2 = 15,9 %). Los participantes completaron un periodo de R_T de 8 semanas con 3 sesiones/semana (fullbody de 6 ejercicios; 3 series x 6-12 repeticiones, 65-80% 1RM y 90 s de descanso). Se midió el grosor muscular de los miembros inferiores y la 1RM en sentadilla (1RMSQ) y press de banca en las semanas 1, 6 y 8 del entrenamiento. Se midió lactato máximo, citocinas (IL-6, IL-10, TNFα), hormonas (GH, IGF-1), % activo de mTOR y mi-ARNs (miR-206, miR-378 y miR-29c) antes y durante los 30 min post-ejercicio después de la primera (S1) y la última (S22) sesión de entrenamiento. El programa de R_T aumentó la 1RM en todos los grupos ($p>0,001$). NH alcanzó una mejora significativa en comparación con N en 1RMSQ (ES = 1,20). El grosor muscular creció de manera similar en N y HH después del periodo de R_T (ES= -0.14; p=1.0), mientras que en NH permaneció cerca del valor previo al entrenamiento (ES= 0.23; p= 0.160). El maxLac se elevó

de manera similar después de la S1 y la S22 en todos los grupos (p=0,895; η^2_p=0,001). Comparado con N, los grupos en HH y NH aumentaron la IL-6 y TNFα en S1 (ES >1,12; p<0,022), volviendo a valores cercanos a los de reposo al final del período de entrenamiento (S22). La GH aumentó en todas las condiciones, aunque no se detectaron cambios en el IGF-1 circulante. El grupo de HH mostró un incremento de moderado a grande en el % activo de mTOR después de la S1 con respecto a N (ES=1.04; p=0.017) y NH (ES=1.34; p=0.002), sin que se alcanzaran diferencias entre grupos en S22. El la condición de NH generó los valores séricos más altos de miR-206 y miR-29c (p<0,020) desde el inicio del entrenamiento. Los grupos de N y HH (p<0,08), mostraron aumentos no significativos de moderados a grandes en el miR-206 (ES>0,76) y una ligera reducción por debajo del valor basal del miR-29c.

Los resultados en hipoxia aguda sugieren que la respuesta metabólica y fisiológica a una sesión de R_T orientada a la hipertrofia están mediadas por los intervalos de descanso entre series y el tipo de hipoxia. La alteración en los niveles circulantes de iones, mioquinas y mi-ARN podría indicar diferencias agudas entre los tipos de hipoxia sobre la activación de la vía de señalización muscular después del R_T. La sobreexpresión aguda del miR-206 en NH podría indicar una tendencia a la preservación muscular e influir en el crecimiento muscular después de períodos de entrenamiento más prolongados. Sin embargo, los resultados obtenidos en este estudio no respaldan el beneficio adicional esperado del RTH en comparación con el RTN en el desarrollo de masa muscular, aunque parece favorecer las ganancias de fuerza. El mayor crecimiento muscular logrado en HH con respecto a NH confirma el impacto del tipo de hipoxia en los resultados. Esto se apoya en la respuesta aguda y crónica de algunos de los biomarcadores evaluados. La investigación futura debería aclarar el efecto del R_T y el papel de la hipoxia en los biomarcadores séricos asociados con el crecimiento muscular y en la influencia de otros factores no estructurales relacionados con el desarrollo de la fuerza muscular.

Research projects

During this doctoral thesis I have participated in the following research projects:

• Effect of a resistance training under different types of hypoxia on the hypertrophy and its link with neuromuscular markers of metabolic stress and associated mechanisms (PGC2018-097388-B-I00-MCI/AEI/FEDER, UE; supervised by Belén Feriche; 72600€; 2019-2022) funded by the Spanish Ministry of Science, Innovation and Universities.

• Effect of hypertrophic training at moderate altitude on the response of markers of metabolic stress and associated muscular growth mechanisms (A-SEJ-246-UGR18; supervised by Paulino Padial; 6400 €; 2020-2021) funded by the Andalusian Regional Government.

• Normobaric hypoxia laboratory (EQC2019-005832-P, supervised by Belén Feriche; 246,419.91€; 2019-2020) funded by the Spanish Ministry of Science, Innovation and Universities, State Research Agency and European Union. Singular Laboratory of the University of Granada.

• Effect of hypertrophic training at moderate altitude on the response of metabolic stress markers and muscle health relationship (B-CTS-374-UGR20, supervised by Belén Feriche; 25,000€; 2021-2022) funded by the Andalusian Regional Goverment, Ministry of Economic Transformation, Industry, Knowledge and Universities.

Research grants

This thesis has been funded by:

• PhD fellowship (FPU pre-doctoral grant FPU18/00686) from the Spanish Ministry of Science, Innovation and Universities. This grant is associated to the research project "Effect of a resistance training under different types of hypoxia on the hypertrophy and its link with neuromuscular markers of metabolic stress and associated mechanisms" (PGC2018-097388-B-I00-MCI/AEI/FEDER, UE).

• FPU Grant for mobility from the Spanish Ministry of Universities, 2023 from June to December (6 months). New York, USA.

Research stays

Stay 1: A one-month research stay (January 2022) was performed at the University of A Coruña (Spain), to learn neuromuscular analysis techniques related to the corticospinal response induced by the transcranial magnetic stimulation as a method to analyze the possible improvement of neuromuscular performance in hypoxia. Supervisor: Associate Professor Gonzalo Márquez, PhD.

Stay 2: A two-month research stay (February-April 2020) was carried out at the University of Extremadura (Spain), to monitor the training and testing procedure during the 8-weeks training under normobaric hypoxia conditions and to learn how to manage hypoxia systems (facial mask and chamber system). Supervisor: Professor Rafael Timón, PhD and Professor Guillermo Olcina, PhD.

Stay 3: A six-month research stay (July-December 2022) was carried out at the Lehman College (University of New York, United States), to study the effect on muscular adaptations under supervised versus unsupervised resistance training and to learn new monitoring ways for resistance exercise. Supervisor: Associate Professor Brad J. Schoenfeld, PhD.

Publications arising from this doctoral thesis

Scientific publications (in reverse chronological order)

Published

► **Benavente, C.**, Schoenfeld, B. J., Padial, P., & Feriche, B. (2023). Efficacy of resistance training in hypoxia on muscle hypertrophy and strength development: a systematic review with meta-analysis. Scientific Reports, 13(1), 3676. Impact factor: 4.997, Q2 in Multidisciplinary Sciences.

► **Benavente, C.**, Feriche, B., Olcina, G., Schoenfeld, B.J., Camacho-Cardeñosa, A., Almeida, F., Martínez-Guardado, I., Timón, R., Padial, P. (2022). Inter-set rest configuration effect on acute physiological and performance-related responses to a resistance training session in terrestrial vs simulated hypoxia. Peer J, 10, e13469. Impact factor: 3.061, Q2 in Multidisciplinary Sciences.

► Timón, R., Olcina, G., Padial, P., Camacho-Cardeñosa, A., Bonitch-Góngora, J., Martínez-Guardado, I., Camacho-Cardeñosa, M., **Benavente, C.**, De la Fuente, B., Feriche, B. (2022) Effects of Resistance Training in Hypobaric vs. Normobaric Hypoxia on Circulating Ions and Hormones. International Journal of Environmental Research and Public Health, 19(6), 3436. Impact factor: 4.614, Q2 in Environmental Sciences.

► **Benavente, C.**, León, J., Feriche, B., Schoenfeld, B. J., Bonitch-Góngora, J., Almeida, F., Perez-Regalado, S. & Padial, P. (2021). Hormonal and Inflammatory Responses to Hypertrophy-Oriented Resistance Training at Acute Moderate Altitude. International Journal of Environmental Research and Public Health, 18(8), 4233. Impact factor: 4.614, Q2 in Environmental Sciences.

► Feriche, B., Schoenfeld, B. J., Bonitch-Góngora, J., de la Fuente, B., Almeida, F., Argüelles, J., **Benavente, C.**, & Padial, P. (2019). Altitude-induced effects on muscular metabolic stress and hypertrophy-related factors after a resistance training session. European Journal of Sport Science, 18, 1-10. Impact factor: 2.781, Q2 in Sport Sciences.

Under submission process

► Feriche, B., **Benavente, C.**, Olcina, G., Padial, P., Argüelles, J., Camacho-Cardenosa, M., Timón, R., León, J. (2023). Influence of the terrestrial vs. simulated hypoxia on biomolecular anabolic signalling response to resistance exercise: A pilot study. European Journal of Applied Physiology. [Submitted]. Impact factor: 3.346, Q2 in Sport Sciences.

Under writing process

► Strength and muscle mass development after a resistance-training period at terrestrial and simulated intermittent hypoxia. [Under writing process]

Conference proceedings

► **Benavente, C.,** Padial, P., & Feriche, B. (2023). Impact of the hypoxia severity on muscle hypertrophy and strength development. II Congreso Internacional Mujer y Montaña. Cumbres en igualdad. Poster.

► **Benavente, C.,** Feriche, B., Schoenfeld, B. J., Bonitch-Góngora, J., de la Fuente, B., Almeida, F., Ortega-Rodríguez, R. & Padial, P. (2020). Acute moderate altitude effect on metabolic response after a hypertrophy-oriented resistance training session. Journal of Strength and Conditional Research, 34 (3), e25. 1st Oral Communication Award in the General Area of the XII International Symposium in Strength Training and IronFEMME Study / I NSCA Spain National Conference in Madrid. Oral Communication.

► **Benavente, C.,** Padial, P., León, J., Schoenfeld, B.J., Bonitch-Góngora, J., Almeida, F., Argüelles, J., Feriche, B. (2021). The effect of a hypertrophy-oriented resistance training session at acute moderate altitude on the relationship between miR-378 and metabolic stress. In: Book of Abstracts, 26th Virtual Congress of the ECSS. European Journal of Sport Sciences. Poster.

► **Benavente, C.,** Padial, P., Feriche, B., Schoenfeld, B.J., Bonitch-Góngora, J., Almeida, F., de la Fuente, B., Ortega-Rodríguez, R., León, J. (2020). The effect of a hypertrophy-oriented resistance training session on acute metabolic and inflammatory and immune markers In: Book of Abstracts, 25th congress of the ECSS. European Journal of Sport Sciences. Poster.

Other contributions (in reverse chronological order)

Scientific publications

Published

► Márquez, G., Colomer-Poveda, D., **Benavente, C.**, Morenilla, L., Alix-Fages, C., Padial, P., Feriche, B. (2023). Altitude-induced effects on neuromuscular, metabolic and perceptual responses before, during and after a high-intensity resistance training session. European Journal of Applied Physiology. Impact factor: 3.346, Q2 in Sport Sciences

► Almeida, F., Padial, P., Bonitch-Góngora, J., de la Fuente, B., Schoenfeld, B. J., Morales-Artacho, A. J., **Benavente, C.** & Feriche, B. (2021). Effects of Power-Oriented Resistance Training During an Altitude Camp on Strength and Technical Performance of Elite Judokas. Frontiers in Physiology, 12, 130. Impact factor: 4.755, Q1 in Physiology

Under submission process

► Rodríguez-Zamora, L., **Benavente, C.**, Petrer, I., Padial, P., Timón, R., Arguelles, J., & Feriche, B. (2023). Hypoxia matters: Comparison of external and internal training load markers during an 8-week resistance training program in normoxia, normobaric hypoxia and hypobaric hypoxia. Journal of Science in Sport and Exercise.[Submitted].

► Pérez-Regalado, S., León, J., Padial, P., **Benavente, C.**, Feriche, B. (2023). Effect of a resistance exercise at acute moderate altitude on muscle health biomarkers. Journal of Science and Medicine in Sport. [Submitted]. Impact factor: 4.597, Q1 in Sport Sciences.

► Coleman, M., Burke, R., **Benavente, C.**, Piñero, A., Augustin, F., Maldonado, J., Fisher, J., Oberlin, D., Vigotsky, A., Schoenfeld, B. J. Supervision during resistance training positively influences muscular adaptations in resistance-trained individuals. Journal of Sports Sciences. [Submitted]. Impact factor: 3.943, Q2 in Sport Sciences.

Under writing process

► Perez-Regalado, S., Petrer, I., **Benavente, C.**, Almeida, F., & Feriche, B. (2023). Impact of the procedure for normobaric hypoxia application in sports performance: systematic review and meta-analysis.

Conference proceedings

► Pérez-Regalado, S., León, J., **Benavente, C.,** Bonitch-Góngora, J., Almeida, F., Padial, P., Petrer, I., de la Fuente, B. & Feriche, B. (2023). Effect of resistance training at moderate altitude on serum muscle damage biomarkers. ECSS Paris 2023. European Journal of Sport Sciences. Poster.

► Pérez-Regalado, S., León, J., **Benavente, C.,** Bonitch-Góngora, J., Almeida, F., Padial, P., Petrer, I. & Feriche, B. (2022). Análisis de la respuesta inmune inducida por el entrenamiento de fuerza en hipoxia moderada. In: Book of Abstracts, XIV International Symposium in Strength Training. Oral Communication.

Awards

► 1st Oral Communication Award at XII International symposium in strength training. Technical University of Madrid and National Strength and Conditioning Association. 2019

► 3rd Prize for the best academic result in Social Sciences Degrees. Ministry of Universities. 2016.

1

Introduction

Resistance training (R_T) is a well-established interventional strategy for increasing human muscle strength and hypertrophy[1,2]. Maintaining high levels of muscle strength and muscle mass is essential to a large section of the population. From a health standpoint, these attributes facilitate the performance of daily living activities and constitute a clear inverse relationship between muscular fitness and mortality [3,4]. From a sports point of view, R_T is essential to improving athlete's performance due to its correlation between muscle cross-sectional area (CSA) and force production[5].

Different training strategies have been developed for optimizing R_T adaptations including load, volume, and inter-set rest interval. However, in the last decades, sports have experienced an increase in the demands imposed on athletes during competitions. In this context, coaches are looking for innovative ways to improve performance efficiently and hypoxic training has become a new strategy to increase the stimulus and outcomes in addition to the use of traditional training[6].

The effect of the hypoxic stimulus has been traditionally studied in endurance training to improve aerobic performance. However, the study of the effect of R_T in hypoxia (RTH) is still in an early stage. The available systematic reviews[7–10] display discrepancies among training methodologies used which makes it difficult to draw firm conclusions about the added benefit of RTH to an equivalent R_T at sea level or normoxic conditions (RTN).

Currently there are different procedures coexisting for reducing the inspired oxygen (terrestrial or simulated) that conceivably could produce different[11] and more effective muscle adaptations between conditions[12]. Additionally, the available literature mainly provides R_T results from intermittent simulated hypoxia being practically non-existent data from terrestrial hypoxia for comparison. Therefore, this doctoral thesis will contribute to fill up the documentary gap on the subject through separate research experiments aiming to 1) Analyze the effect of the R_T exercise under the two types of acute and chronic moderate hypoxia conditions (terrestrial vs. simulated) on both the physiological response and the muscle and strength performance markers; and 2) to determine the

potential benefit of RTH on muscle hypertrophy.

The following subsections will introduce the main theoretical background supporting the experimental research conducted in the present doctoral thesis.

1.1.—Resistance training

Strength is the most decisive conditional physical capacity in the context of sports performance, leisure or rehabilitation[13] because it is involved in the development of all movements[14]. At a general level, the term "*force*" can be defined as something which changes or tends to change the state of rest or motion in matter. However, from a sport training point of view, it is defined as the ability of a muscle or group of muscles to generate muscle tension under specific conditions [15]. Thus, the ability to train strength has been widely studied and documented over the past years[16,17].

R_T is an exercise intervention that is used to improve muscle mass, strength and bone mass, generate bigger quality, increase health-related quality of life and increase resting rate[18,19]. Athletic success and the overall well-being need the implementation of a correct R_T program to optimize strength and muscle mass[20]. These improvements are influenced by the structure of the training program[21].

It has been reported that most of the strength gains during the initial weeks of R_T are due to adaptations in facilitatory and inhibitory neurological pathways acting at different levels in the nervous system[22,23]. Thus, muscular strength can be improved in the absence of hypertrophy[24] by enhancing the brain's ability to recruit specific motor units to contract and produce a desired movement[25]. However, muscle hypertrophy is highly associated with long-term strength enhancement in resistance trained individuals[26], but it could not be possible if the central system does not recruit muscles effectively to produce force[5] (e.g., greater recruitment, rate of discharge)[27]. Thereby, optimizing the R_T programming aiming to increase muscle hypertrophy needs the proper manipulation of variables such as muscle actions, load/intensity[28], volume[29], exercise selection and order, inter-set rest periods[30], and frequency[31], considered fundamental components for maximizing neural and morphological adaptations[1]. Nowadays, how

to optimize R_T protocols to maximize muscle mass and strength remains to be fully determined.

1.1.1 Hypertrophy

The positive correlation between muscle force and CSA[32] makes hypertrophy the primary goal for professional and recreational athletes. Hypertrophy is defined as an increase in muscular size, which can be achieved through exercise[33]. Traditionally, hypertrophy R_T is characterized by moderate load, high total volume load and short inter-set rest intervals[34] although their effects may vary depending on the manipulation of the training variables[35].

In adult skeletal muscle, the capacity of regeneration is critical for the repair and functional maintenance of skeletal muscle. The regeneration of adult muscle is based on the activation of satellite cells, which are mononuclear progenitors of skeletal muscle and are located between the sarcolemma and basal lamina[36]. Satellite cells are activated and contribute to hypertrophy when an acute stimulus is involved, after strong or unaccustomed exercise or ultimately, when some form of muscle damage occurs[37]. After injury, the regeneration of muscle occurs in three stages: in the first stage, inflammatory cells infiltrate into damaged sites, and necrotic fiber fragments are removed; in the second stage, satellite cells are activated and proliferate into myoblasts, thereafter differentiating and fusing to form new muscle cells (myoblasts) and replace damaged fibers[38]; the last stage involves the maturation of newly formed fibers and the remodeling of damaged muscle[39].

1.1.2 Types of muscle hypertrophy

The increase in muscle mass can be achieved through muscle hypertrophy or hyperplasia. Nevertheless, both mechanisms must be considered in a distinct and separated form (Figure 2):

Hyperplasia: It is an increase in the number of fibers within a muscle. It is well accepted that the number of myofibers per muscle increases during the early stages of developmental growth[40] and the overall effects on muscle cross-sectional area appear to be lacking[41] or minimal[42] in adult skeletal muscles.

Hypertrophy: It occurs when the contractile elements enlarge and the extracellular matrix expands to support

growth. Contractile hypertrophy can occur either by adding sarcomeres in series or in parallel.

Taking into account these concepts, the increase in muscle mass in adults is the result of an increase in the size of skeletal muscle, which is accompanied by an increase in mineral, protein, or substrate abundance[43]. The majority of muscle hypertrophy caused by a traditional R_T program results from an increase in the total number of sarcomeres in parallel and by an increase in the size and amount of the myofibrillar contractile proteins actin and myosin[44], increasing the capacity of the muscle to produce force[5]; whereas the addition of sarcomeres in series happens when muscle is forced to adapt to a new functional length such as an immobilization[45] or at certain types of exercise such as isolated eccentric contractions[46]. When the size of the muscle is increased, there is not only myofibrillar hypertrophy, but also connective tissue and sarcoplasmic fluids.

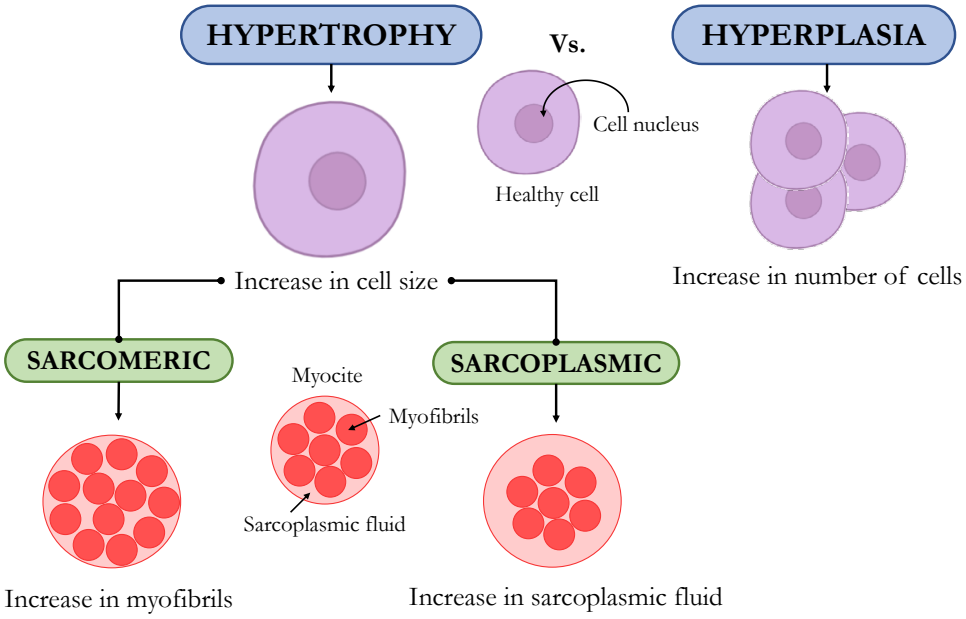


Figure 2 — Types of muscle hypertrophy and differences between hyperplasia.

This has been termed as "*sarcoplasmic hypertrophy*" and is based on anecdotal observations suggesting that bodybuilders have bigger muscles than powerlifters but they are not so strong[47]. Thus, increases in sarcoplasmic hypertrophic are thought to be training specific and because of differences in training methodologies[48].

1.1.3 Mechanisms responsible to the hypertrophic response

Mechanical tension, muscle damage and metabolic stress are the 3 primary factors hypothesized to be related to the hypertrophic response to R_T[2] (Figure 3):

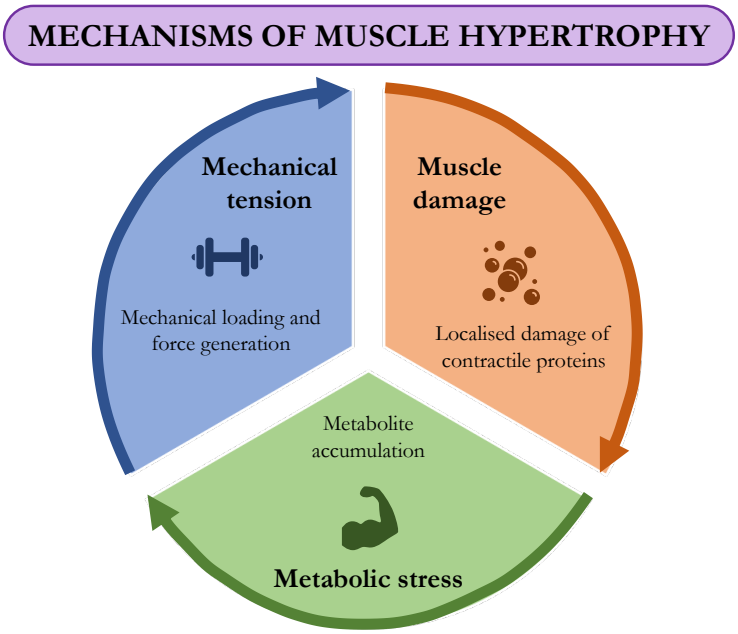


Figure 3 — Mechanisms of muscle hypertrophy.

1.1.3.1 Mechanical tension

Mechanical tension produced both by force generation and stretch, is generally considered the most relevant factor for muscle growth[49,50]. When a muscle is subjected to mechanical stimuli (both the magnitude (load) and the time under tension), the body initiates a process called mechano-transduction, disturbing the integrity of skeletal

muscle and causing chemical signals that regulate anabolic and catabolic processes through a variety of enzymatic pathways[51]. This process appears to accelerate the protein synthesis rate, probably through a cascade activation that involves growth factors, cytokines, or stretch-activated channels[50,52]. Evidence suggests that the activation of cellular receptors is regulated via the protein kinase B/mammalian target of rapamycin (AKT/mTOR) pathway or indirectly via phosphoric acid[49]. However, the upstream signaling pathways that promote protein synthesis remain controversial[53]. Although mechanical tension could be essential for the development of muscle mass, evidence suggests that it is not the only cause for muscle hypertrophy[54].

1.1.3.2 Muscle damage

R_T can damage the skeletal muscle tissue, primarily from the performance of unaccustomed exercise, and from the severity of the exercise, modulated by the type, intensity, and duration of training[55]. Damage can be specific to just a few macromolecules of tissue, or result in large tears in the sarcolemma, basal lamina, and supportive connective tissue, inducing injury to contractile elements and the cytoskeleton[56] R_T is also theorized to generate a hypertrophic response under certain conditions (such as eccentric exercise or higher levels of tension)[57].

When the body perceives muscle damage due to membrane deformation, a calcium (Ca^{2+}) homeostasis disruption occurs and triggers an acute inflammatory response[58]. This response causes a migration of the white cells (macrophages, lymphocytes and neutrophils) to the damaged area to maintain the fibers' structure. Macrophages remove the cellular debris and produce cytokines that are believed to lead the release of various growth factors that regulate satellite cell proliferation and differentiation [45], promoting the muscle growth[59,60].

1.1.3.3 Metabolic stress

The accumulation of metabolites (i.e., lactate, inorganic phosphate (Pi), hydrogen ion (H^+), creatine phosphokinase (CPK), and others[61] from intense exercise has been called "*metabolic stress*"[54]. Metabolic stress in R_T occurs as a result of anaerobic metabolism and is maximized during exercise when anaerobic glycolysis is the main source of

energy. This metabolite accumulation facilitates exercise-induced hypertrophy by the activation of a complex cascade of anabolic and catabolic signaling pathways to favor synthesis over degradation. A prolonged shift of muscle protein turnover toward synthesis rather than breakdown results in skeletal muscle hypertrophy[62]. It also has been hypothesized that metabolic stress inducing muscle hypertrophy is mainly related to the increased motor unit's recruitment provoked by muscle fatigue in order to maintain the mechanical forces when a set is close to failure[63].

The main pathways that have been identified as particularly important to muscle anabolism include mTOR, mitogen-activated protein kinase (MAPK), and various calcium-dependent pathways, amongst others. Although the precise mechanisms between them have not yet been fully elucidated, evidence suggests that they may be synergistic rather than redundant key regulator steps[64].

The potential hypertrophic role of exercise-induced metabolic stress has been addressed by the literature over the past years:

Routines involving the performance of multiple sets of 6-12 repetitions per set (60-80% RM) with relatively short inter-set rest intervals. These routines performed by bodybuilders have been found to induce more metabolic stress than regimens employed by powerlifters with higher-intensity routines[65].

The combination of R_T with blood flow restriction (BFR). Although BFR is carried out at low intensities (generally <40% RM) while using a pressure cuff to induce ischemia, the evidence supports gains in muscle mass by the stimulation of anabolic signaling and protein synthesis[66].

R_T carried out in a hypoxic environment (see subsection 1.3.). Although the current literature shows controversy about the beneficial effects of hypoxia over a traditional training[7–10], some studies observed a significantly higher metabolic stress in H compared to N determined by blood lactate levels[67], growth hormone[67–70] and circulating ions (Na^+, Cl– or H^+)[71].

Several factors have been theorized to mediate hypertrophic adaptations from exercise-induced metabolic stress (Figure 4):

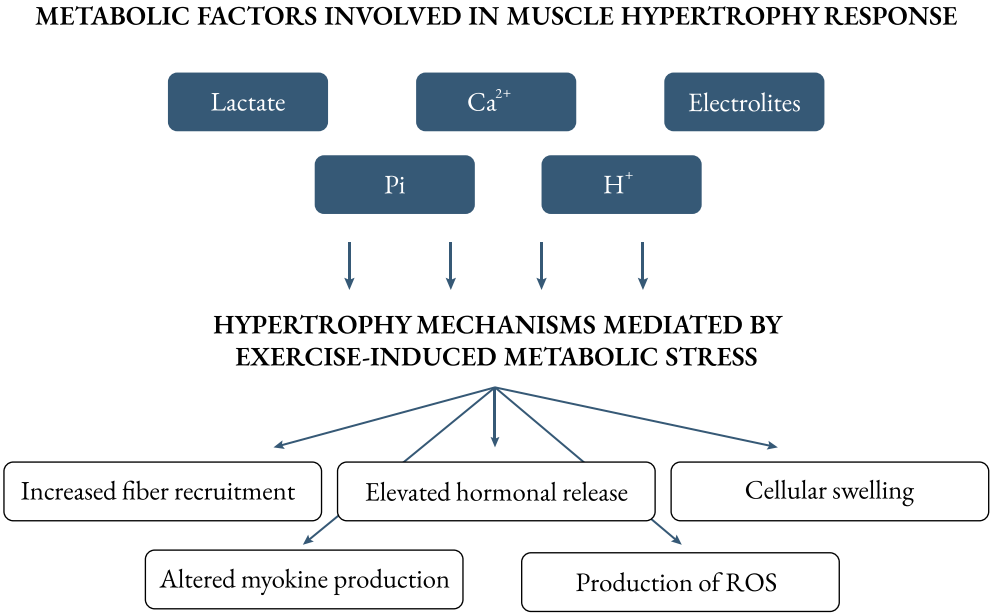


Figure 4 — Hypertrophy mechanisms mediated by metabolites. Adapted from Feriche et al. [8] and Schoenfeld [64].

a — Fiber recruitment.

Motor units are recruited in an orderly fashion, always from small to large, to sustain muscle contraction when the training intensity increases[72]. Thus, as more motor units are recruited, higher mechanical forces will be produced by the muscles[73,74]. Neural adaptations are predominant in strength gains during the early stages of R_T[75]. Hence, one of the possible explanations to strength gains in untrained subjects is increases in muscle recruitment. In this line, the central nervous system is able to modulate the mechanical forces produced by each muscle through sending them action potentials at different rates during simple or complex exercises[76]. Numerous investigations have noted that this neural drive, measured via the amplitude of muscle electromyography (EMG) signals, is also increased following a period of traditional R_T[77].

It has also been hypothesized that metabolic stress enhances fiber recruitment. Although there is speculation that an H^+ accumulation activates more glycolytic (i.e., type II) motor units to maintain the same level of

force generation[78], the mechanisms are not well stablished. However, metabolite accumulation can also stimulate group III and IV afferents[79], a reflexive net inhibitory effect on the α-motor neuron, promoting the recruitment of additional high-threshold motor units to maintain force[80,81] and protect against failure. Otherwise, several investigations have reported increased levels of muscle activation during resistance exercise with BFR[82,83]. The hypoxic condition and the associated increase of metabolite accumulation during BFR exercise, and hence after a systemic hypoxic stimulus[84], may provide an additional stimulus to muscle mass development.

b — Elevated systemic hormonal production.

Hormones play an integral role in regulating the anabolic responses to resistance exercise[64]. R_T regulates muscle protein turnover by modifying the anabolic (growth hormone (GH), insulin-like growth factor (IGF)-1 and testosterone) and catabolic (cortisol) responses to a workout by altering the balance between anabolism and catabolism [85]:

» *IGF-1*

IGF-1 is an anabolic and metabolic hormone released by the liver into the systemic circulation and also locally produced in tissues and cells[86]. The anabolic effects include satellite cell activation, proliferation, survival, and differentiation[87]. The metabolic effects include stimulation of the protein synthesis, increment of free fatty acid utilization, and enhancement of insulin sensitivity[88]. Local production of IGF-1 from liver is under GH regulation and is associated with mechanical-stretch mechanisms and the recruitment of motor units[89].

IGF-1 released from the muscle, known as mechano-growth factor (MGF), is rapidly expressed following mechanical loading and is thought to start the post-exercise hypertrophic response and facilitate local repair of damaged tissues[90]. Some research suggests the IGF-1 as the primary physiological regulator of muscle mass[91]. The elevation of IGF-1 has been shown to be proportional to the gains in muscle strength following R_T[92]. IGF-1 carried out signaling through multiple anabolic cascades, including PI3K-Akt-mTOR[93] and several

calcium-dependent pathways[94], directly mediating synthesis of muscle proteins (Figure 5). Although the effects of IGF-1 on muscle hypertrophy are not clear due to the milieu of anabolic signaling to skeletal muscle, it is believed that the primary hypertrophic role of IGF-1 is to activate, repair and promote the proliferation of satellite cells when skeletal muscle cells are stimulated or damaged by external factors[95].

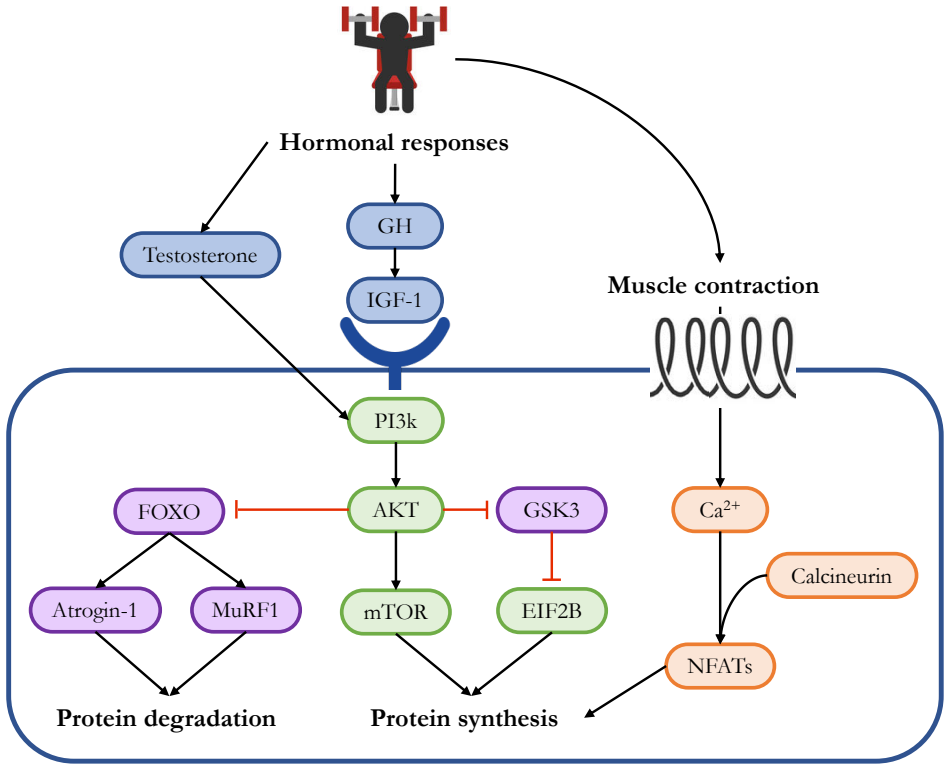


Figure 5 — Signaling pathways regulated by testosterone, growth hormone (GH) and insulin-like growth factor 1 (IGF-1) induced by resistance exercise. AKT: protein kinase B; Ca^{2+}: calcium; FOXO: forkhead box O; GSK3: glycogen synthase kinase-3; eIF2B: eukaryotic initiation 2B; mTOR: mammalian target of rapamycin; MuRF1: muscle ring finger-1; NFATs: nuclear factor of activated T-cells; PI3k: phosphatidylinositol 3-kinase. Adapted from[64,96].

Circulating IGF-1 can vary due to temporal frame of measurement or differences between protocols [34,97]. In this line, greater elevations of circulating IGF-1 have been found after a hypertrophy-type routine compared to high-intensity protocols[65,98] or following BFR exercise[99,100]. However, serum levels of IGF-1 may not be a good reflection of its local effects[101], especially in skeletal muscle, capable of producing the hormone itself. Indeed, circulating IGF-1 levels have even been shown to decrease during periods of active muscle building, likely due to a redistribution of IGF-1 from the circulation system into the muscle[102]

» *Testosterone*

Testosterone is an anabolic-androgenic steroid hormone which is synthesized from cholesterol in the Leydig cells of the testes under control of the hypothalamic-anterior pituitary-gonadal axis in men, and via conversion from progesterone in the ovary and adrenalzona fasciculata in women[103]. It is taken up by the muscle through androgen receptors, stimulating subsequent myocellular signaling[104,105] and altering the expression of genes involved in the regulation of skeletal muscle structure, fiber types, metabolism and transcription[106].

The response of testosterone in men and women[88,105] and the associated anabolic effects[107,108] have been extensively reviewed. Studies suggest the androgens play an important role in mediating skeletal muscle protein synthesis and reduce protein catabolism and autophagy[109]. In addition, testosterone appears to potentiate the release of other anabolic hormones such as GH[85] and IGF-1[110], as well as moderate satellite cell activation and proliferation[60], leading to increase muscle strength, power, endurance, and hypertrophy in a dose-dependent manner[88].

Even though the literature has depicted significant correlations, more pronounced in strength athletes[111], between training-induced elevations in testosterone and increases in muscle CSA[112], a causal relationship between acute testosterone production and hypertrophy has yet to be established. Conflicting results have been found after hypertrophic-oriented R_T programs on testosterone. Several studies display greater post-exercise testosterone elevations[113–115] compared with routines not focused on metabolic

stress, while others failed to find such changes[61,116,117]. Moreover, BFR, characterized by the production of high levels of metabolites, is not associated with significant post-exercise elevations in testosterone[61,99,118], questioning the role of hormones in the metabolic stress-induced hypertrophic response.

It has to be taken into consideration that the magnitude of the testosterone release is affected by many factors including gender, age, training experience, the demands of the protocol and nutritional status[88]. Further investigation is needed to reach definitive conclusions due to the inconsistent results.

» *Cortisol*

Cortisol is a glucocorticoid steroid hormone systematically controlled by the hypothalamic-pituitary-adrenal (HPA) axis to maintain glucocorticoid homeostasis in general and to regulate energy homeostasis and metabolism in the skeletal muscle[119].

Cortisol contributes to muscle remodeling through muscle protein synthesis inhibition and stimulating protein degradation[112]. The intensity and volume of resistance exercise performed influences the response of the HPA axis. The greatest acute cortisol elevations are detected when the overall stress is high (involving large muscle mass, with moderate to high loads, and short rest intervals)[120–122]. During exercise, cortisol increases the availability of metabolic substrates. After exercise, the tissue sensitivity to glucocorticoids increases and serves to counteract muscle inflammation, cytokine synthesis, and muscle damage[123], preparing the body for the next bout[124,125].

However, another mechanism to protect tissues and cells from the deleterious effects of exercise-related cortisol secretion implies inactivation, which appears to be affected by overtraining[126]. Likewise, high levels of cortisol may decrease skeletal IGF-1 synthesis[127], increase catabolic growth factors, such as myostatin[128,129], and activate the expression of genes involved in atrophy, such as FOXO, atrogin-1 and MuRF-1[130,131].

In already trained individuals, a single bout of exercise attenuates the cortisol respons[132] and reduces the resting cortisol concentrations[133] which may facilitate ongoing muscle

adaptations. For this reason, cortisol responses depend on workout design, nutrition (carbohydrate and amino acid administration)[134], genetics, training status and type of RT[135].

» *GH*

GH is a superfamily of different molecular isoforms, released from the anterior pituitary gland, that mediates physiological actions during recovery in response to exercise[136,137].

The type of exercise may also have an influence on GH release, although the unifying thought is the influence of pH and H^+ ions status on it[138]. When blood lactate concentration is elevated beyond the anaerobic threshold or after an intense R_T session, the pH decreases and rises the release of H^+ to the bloodstream, highly affecting the GH response[116,139]. This is supported by the positively correlation between blood lactate and GH, especially when using short rest periods (<90 s) between R_T sets[88,140].

GH primarily carries out muscle anabolism by potentiating the release of IGF-1 in liver and muscles[141], to coordinate muscle growth and repair following resistance exercise[140]; although some researchers dispute this theory and postulate additive hypertrophy effects of GH and IGF-1[142]. GH also appears to have a synergistic effect on testosterone-mediated protein synthesis[105]. However, it is not clear whether an increase of GH, testosterone and IGF-1 circulating levels does in fact mediate an enhanced hypertrophic and strength response[143].

Finally, studies conducted with trained population on the subject are lacking. Thus, it still has to be clearly elucidated if those with previous training experience respond differently to acute exercise-induced hormonal output compared with untrained subjects[144]. Modest increases in muscle hypertrophy could potentially be meaningful for certain populations, particularly, strength athletes[64].

c — Alteration in local cytokines.

The role of cytokines has received growing interest since the discovery of the adipose tissue as an endocrine organ capable of secreting substances to modulate metabolic processes[145].

Cytokines are a group of proteins and glycoproteins that contribute to different biological and physiological

mechanisms, such as blood pressure, inflammation and cellular metabolism[146,147]. Cytokines regulate, modulate, and orchestrate biological systems in hematopoietic, immune, and non-immune cells to produce development, differentiation, function, growth, and regulation, throughout the employment of paracrine and autocrine signaling pathways[148,149]. The homeostasis across the systems of the human body is provided by an influent balance between anti-inflammatory and pro-inflammatory cytokines, considered as part of the acute (local or systemic) response to an infection or tissue injury (Table 1). They are released at the site of inflammation and facilitate an influx of lymphocytes, neutrophils, monocytes, and other cells that participate in the clearance of the antigen and healing[150].

The cytokines only produced and released from skeletal muscle cells in response to muscular contractions are called myokines. During exercise training, the synthesis of various cytokines and myokines within skeletal muscle results in potential hypertrophic adaptations[151–153]. It is speculated that metabolic stress may mediate muscle hypertrophy by either upregulating anabolic myokines and/or downregulating catabolic myokines.

There are some cytokines playing important roles in muscle development after a R_T:

»*Interleukins*

Interleukins (ILs) are a group of cytokines that normally act on immune cells to regulate immune and inflammatory responses. However, it is now becoming clear that at least a subset of interleukins could be implicated in skeletal muscle repair and regeneration such as the IL family[154].

Interleukin (IL)-6 has been shown to play an important role in myogenesis, stimulating proliferation[155,156], and differentiation[157]. It has been postulated that exercise-induced metabolic stress may also stimulate the IL-6 production[145], founding a positive relationship between circulating IL-6 and muscle hypertrophy after acute resistance exercise[158]. The acute increase in circulating cytokines following resistance exercise may play influence on activating satellite cells and contribute to muscle growth[159].

In addition to IL-6, other interleukins are also associated with exercise. Taking into account muscle growth and regeneration, an increased protein expression of IL-8 and IL-10 was found following a bout of resistance exercise[154]. Moreover, the ablation of specific interleukins demonstrated impaired muscle growth after injury (IL-4)[160] or during periods of overload-induced hypertrophy (IL-6[151] and IL-10[161]). IL-4 is also increased during myoblast differentiation[162]. Additionally, the administration of IL-15 results in an increase in muscle weight[163] and its overexpression is related to a greater accumulation of myosin heavy chain compared to control[164,165].

However, these results do not directly link interleukins to muscle growth or directly compare the effect of cytokines after traditional hypertrophy or strength-oriented routines. Thus, it is difficult to draw firm conclusions about whether metabolic stress influences muscle hypertrophy by altering cytokine production. This topic could be a focus for future research.

» *Tumor necrosis factor-α (TNF-α).*

TNF-α is a cytokine that mediates cellular inflammatory signaling pathways. It is produced by activated macrophages but also by muscle cells during the regeneration process[166], regulating proliferation and differentiation of satellite cells and myoblasts[167].

Table 1 — Cytokines, exercise and myogenesis. Type or function of each cytokine, the tissue in which the interleukin was measured and the role in myogenesis. Adapted from Joanisse & Parise[159]

Cytokine	Type/Function	Tissue	Role in myogenesis
IL-4	Anti-inflammatory	Muscle	Proliferation, differentiation, migration
IL-6	Anti/pro-inflammatory	Plasma, muscle	Proliferation, differentiation
IL-8	Anti-inflammatory	Muscle	Acute increase following exercise
IL-10	Anti-inflammatory	Muscle	Proliferation
IL-15	Pro-inflammatory	Plasma, muscle, connective tissue	Increase in muscle weight when administered to mice
TNF-α	Mediator in inflammation and apoptotic signaling pathways	Plasma/macrophages	Proliferation, differentiation, regeneration

Some studies assessed the circulating levels of TNF-α after exercise indicating an elevation immediately after RT[168] and 24 hours[169] or 48 hours after an eccentric exercise session[170]. In endurance training, there are no differences in TNF-α 48 hours after a bout of downhill running[171]. Also, TNF-α deficiency shows a decreased myoblast determination protein 1 (MyoD) expression and myogenesis[172]. Thus, different exercise protocols, alongside distinct measurement methods, and targeted population might explain differences among these studies and cytokine changes.

»*Myostatin*

Myostatin is a myokine member of the transforming growth factor-β (TGF-β) superfamily, which inhibit skeletal muscle growth[173]. Myostatin expression restrains both myoblast proliferation through PI3K/Akt/GSK-3β signaling pathway[174] and differentiation by downregulating expression of MyoD, myogenin, and Myf5[175,176]. Myostatin has also been shown to maintain the satellite cells in a quiescent state[177,178]. Recent studies in humans suggest that myostatin is a negative regulator of satellite cells in response to an acute bout of resistance exercise[179]. The

myostatin inhibition represents a myogenic stimulus to muscle hypertrophy in response to prolonged RT[180].

»*Irisin*

Another myokine whose circulating levels are associated with exercise training is the irisin[181]. Irisin is regulated by peroxisome proliferator-activated receptor (PPAR)-γ coactivator- 1α (PGC-1α) and it is shown to increase adipocyte metabolism, which translates into body weight reduction, oxygen consumption, and enhances glucose metabolism[182]. The receptor and the role of irisin in myogenesis are mostly unknown. However, some studies revealed an increased muscle fiber thickness under an irisin exogenous treatment[183], which is positively correlated with biceps circumference and IGF-1 levels in humans[184]. Additionally, irisin regulates FNDC5 and PGC-1α, myoblast fusion and stimulates muscle growth-related genes[185].

The role of irisin after acute and chronic exercise training has been well documented in the last years. Acute exercise is associated with significant post-exercise increase in irisin concentration[181] while chronic exercise revealed a non-significant or even a decreased irisin levels[186]. However, these results should be used with caution due to the lack of specificity of the analysis procedure and/or the post-exercise time point of assessment (from 30 minutes to 2-7 days)[181].

d — Heightened production of reactive oxygen species (ROS).

The term ROS include both oxygen radicals and non-radicals oxidizing agents. ROS production is influenced by environmental stress and aging[187]. During exercise, contracting muscles are an important source of acute ROS production[188]. Although the mechanisms of action of the ROS have not been elucidated, they can function as key cellular signaling molecules in the response to exercise, promoting muscle growth via enhanced MAPK signaling[81].

e — Cell swelling.

This phenomenon involves an increase in intracellular hydration. This mechanism has yet to be fully determined although it seems the cell initiates a signaling response when the increased pressure against the cytoskeleton is perceived as threatening

cellular integrity[189]. To reinforce the cell ultrastructure, research indicates that anabolic functions are carried out in a mTOR-independent fashion[190], suggesting the MAPK as the primary mediator of swelling-induced anabolism[191]. Cell swelling could also induce muscle growth through the proliferation and fusion of satellite cells[192]. R_T seems to maximize the cell swelling process with the resultant lactate accumulation[193]. Glycolysis produces cell swelling as a result of the lactate accumulation which acts as a contributor to osmotic changes in skeletal muscle. Consequently, this acidic environment caused by the metabolite accumulation promotes a growth response, although the potential anabolic mechanism of cell swelling is still unclear[194].

Although there is compelling evidence that metabolic stress also contributes to hypertrophic adaptations[66], a large body of evidence states the mechanical stress as the primary driving stimulus in post-exercise muscle growth[64]. Likewise, both mechanisms can work in tandem, making it difficult to measure separately the effects of one from other on muscle hypertrophy[54].

f — Other involved pathways.

» *Gene expression: microRNA.*

Regulation of gene expression includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (protein or RNA). Gene expression can be modulated from transcriptional initiation to RNA processing, and to the post-translational modification of a protein. Among them, microRNAs are small, endogenous, non-coding RNA molecules which are involved in the post-transcriptional regulation of many genes[195] by mRNA degradation[196], translational repression[197], or via the combination of both processes. Initially, genes encoding miRNAs are transcribed in the nucleus and managed through a series of biogenesis steps[198] (Figure 6).

Interestingly, miRNAs are stable and detectable in blood since they are protected from RNase activity[199]. miRNAs can be secreted into the bloodstream by different organs including vascular endothelial cells, skeletal muscle[200], heart[201], liver[202] and brain[203].

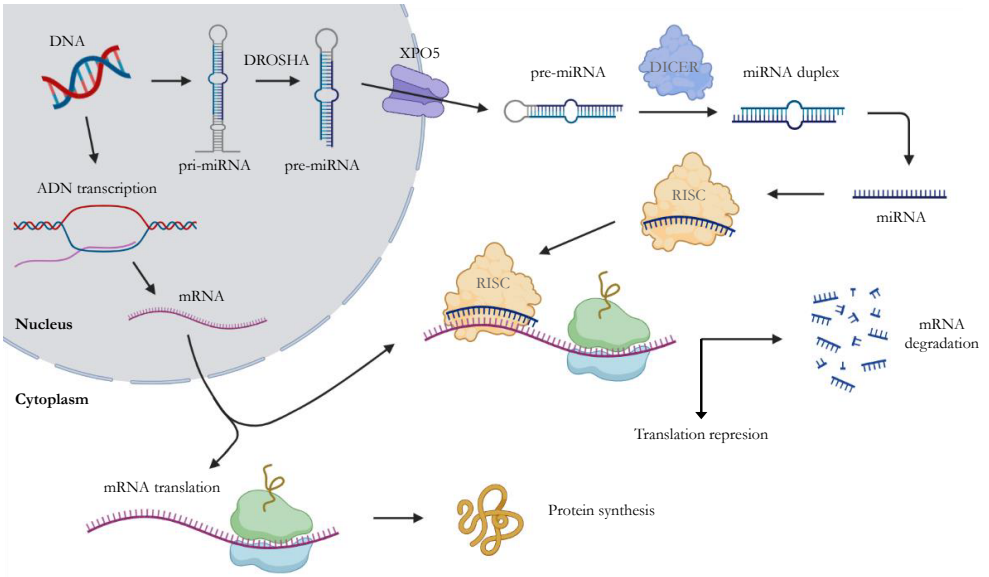


Figure 6 — MicroRNA biogenesis and mechanism of action.

Some circulating miRNAs might reflect physiological or pathophysiological processes occurring in these tissues. Due to their stability in blood, miRNAs have been considered as promising biomarkers[204]. Most of the available studies that analyze miRNAs expression have been carried out in animals using biopsy. However, recent studies in humans used both biopsy and serum/plasma for detection.

Recently, a variety of miRNAs (i.e., miR-1, -21, -23a, -29, -31, -126, -133a/b, -181, -206, -378, -486 and -696) have been shown to regulate important biological processes in muscle, such as growth, metabolic adaptation, and repair in response to exercise and training (Table 2). During skeletal muscle development, some miRNAs are specifically expressed in skeletal muscle cells (also called myomiRs such as miR-206, miR-1, and miR-133) while others are non-muscle specific but differentially expressed around the differentiation process[205]. These miRNAs can target several myogenic transcription factors (Pax, MyoD, myogenin, etc)[206], involving skeletal muscle regeneration or myogenesis, by satellite cells activation, proliferation and differentiation[207].

Table 2 — miRNAs and target implicated in the development of skeletal muscle. Adapted from Kirby and Mccarthy [197] & Nie et al. [198].

miRNA	miRNA target (s)	Expression pattern	Function in myogenic differentiation
miR-1	HDAC4, Pax7	Heart, skeletal muscle	Promote differentiation Inhibit proliferation
miR-23a	Myh	Not muscle-specific	Inhibit differentiation
miR-29c	HDAC4, YY1, Akt3	Not muscle-specific	Promote differentiation Inhibit proliferation and fibrosis
miR-31	Myf5	Not muscle-specific	Maintenance of quiescence/stemness
miR-133	SRF, UCP2	Heart, skeletal muscle	Promote proliferation Inhibit differentiation
miR-181	Hox-A11	Not muscle-specific	Promote differentiation
miR-206	Cx43, Pax3/7	Skeletal muscle	Promote differentiation Inhibit proliferation
miR-378	MyoR	Not muscle-specific	Promote differentiation
miR-486	Pax7	Heart, skeletal muscle	Promote differentiation

In muscle hypertrophy, the IGF1/PI3K/AKT/mTOR signaling pathway[210] is the most important process involved in skeletal protein synthesis. The activity of IGF1/PI3K/AKT/mTOR signaling is controlled by several feedback loops, positive and negative, that regulate muscle growth[210]. Indeed, various miRNAs, directly and/or indirectly, regulate this pathway[211] depending on the nature of physical exercise or training[212]. In the section can be found some miRNAs frequently studied concerning muscle mass development:

The miRNAs whose expression is restricted to skeletal myoblasts and cardiac tissue during embryonic development and muscle cell differentiation are called MyomiRs (miR-1, miR-133 and miR-206)[213]. MyomiRs are involved in the regulation of Pax7 and are themselves regulated by downstream genes such as MyoD[214] which control muscle hypertrophy, atrophy,

myogenesis, and apoptosis via cell cycle regulation[215].

miR-1 and -133 can be expressed in either skeletal muscle or cardiac muscle. In skeletal muscle, their expressions significantly increase during myogenesis but reduce in muscle during hypertrophy in response to RT[216] whereas the IGF-1 protein level significantly increases[217]. Therefore, it has been proposed that miR-1 and -133a target IGF-1/IGFR in the IGF-1/PI3K/AKT pathway, thereby reducing their expression and leading to signaling pathway activation.

miR-206 is a myomiR whose expression is restricted to skeletal muscle. During skeletal muscle development, Pax3 and Pax7, target genes of miR-206, are able to prevent early differentiation of myoblasts. An increased expression of miR-206 is produced when myoblasts are not decreased in time and the inhibition of Pax3 and Pax7 prompted myoblasts to successfully enter into terminal differentiation[205]. Recent studies revealed that miR-206 promotes differentiation[218], participates in skeletal muscle regeneration following injury in mice[219] or displays an upregulation in athletes with a muscle fast-twitch fibers predominance[220]. In tilapia, inhibition of miR-206 in skeletal muscle promotes body growth and an increase in IGF-1 expression[221]. Moreover, a decreased miR-206 expression is linked to upregulation of myostatin mRNA in murine models[222]. Thus, the functions listed for miR-206 in skeletal muscle development demonstrate its positive roles for myogenesis, despite its negative role in the differentiation process on occasion in order to successfully make the myoblast enter into the fusion stage[205].

miR-29 and miR-31 also participate in myogenesis. Although the mechanism is not fully understood, miR-29c is found to specifically downregulate Akt3 in vitro study in mouses, thereby potentially reducing proliferation and promoting differentiation of myoblasts in skeletal muscle development[223] and the increase of the muscle mass[224]. However, the research about miR-29c expression is still scarce and additional investigation is needed to address the regulatory role of this miRNA in skeletal muscle.

miR-378 is a non-muscle specific miRNA whose overexpression increases the transcriptional activity of the

MyoD, purportedly by repressing its antagonist (MyoR)[225], which in turn may enhance processes related to muscle hypertrophy[226]. miR-378 expression via a biopsy has showed reduced basal levels in low responders whereas its expression is unchanged in high responders after a R_T in healthy individuals[227]. The change in miR-378 expression has also been correlated with lean mass gain after R_T[227]. Moreover, miR-378 levels seem to increase just after an acute bout of resistance exercise[228] but remains at basal levels 1- and 3-hours post exercise[229], which suggests that maintenance of miR-378 expression may be necessary for promoting resistance exercise-induced muscle growth.

1.1.4 Training variables and muscle hypertrophy

The key factors or training variables essential for maximizing exercise-induced muscle hypertrophy are the combination of the load, sets, repetitions, rest, velocity of movement and exercise selection. The proper manipulation of these variables is largely discussed on the literature drawing the following conclusions:

1.1.4.1 Load and volume.

Training load and volume have a significant impact on muscle hypertrophy and constitute the most important exercise variable for stimulating muscle growth[230]. Training load refers to the amount of weight assigned to an exercise set[231]. The training load can be expressed as a percentage of 1-repetition maximum (1RM) or the greatest amount of weight lifted with correct technique and equates to the number of repetitions that can be performed with a given weight[232]. Volume load describes the total amount of work performed within a training session (Absolute volume load = sets x repetitions x weight lifted (kg))[233]. The volume load is easy to implement and is largely related to measures of internal load and physiological stress during R_T[234]. However, this approach has some limitations between individuals, given that this measure does not reflect the relative intensity of the loads lifted by each individual. Thus, the relative volume load can correct the inter-individual variations (Relative volume load =sets x repetitions x %1RM)[235]. Nevertheless, the difficulty associated with performing the prescribed number of repetitions in

a set at a given load constitutes a limitation which can be corrected calculating the intensity and volume loads for each set relative to the predicted RM weight for the prescribed number of repetitions (Volume load relative to specific RM=sets x repetitions x %RM for repetition range used)[235].

When the training goal is focused on maximum strength development, heavy loads are frequently used (1-3 RM and/or 3-8 RM); moderate loads for hypertrophy (8-15 RM) and low loads for muscle endurance (>20 RM)[236].

A moderate range of 6-12 reps is believed to optimize the hypertrophic response[237], due to factors associated with metabolic stress. In addition, a moderate repetition range and multiple-sets protocols maximize the acute hormonal response of exercise (elevated GH and testosterone) compared to using low repetitions[113,121] and augment cell swelling by the accumulation of metabolic byproducts[193]). Therefore, R_T programs targeting muscular strength and hypertrophy are best served by moderate to heavy loads (6-15 RM)[35], but some factors, as the training status, must be taken into consideration when prescribing exercise[238].

1.1.4.2 Exercise Selection and order.

Exercise selection involves choosing exercises for a R_T program[231]. Several classifications are used for different types of exercises based on the size of the muscle area involved. Resistance exercises can be classified according many different criteria. The most common classification is multi-joint or single-joint exercises considering the number of joints involved in the exercise. The evidence supports the inclusion of both multi-joint and single-joint exercises in hypertrophy routines[239]. Multi-joint exercises recruit large amounts of muscle mass, increasing the anabolic response and producing larger increases in GH and testosterone[240] while single-joint allow for a greater focus on specific muscles.

Exercise order refers to a sequence of resistance exercises performed during the training session[231]. Traditionally, multi-joint exercises and/or those involving large muscle mass are performed before the single-joint and/or small muscle exercises because they demand higher intensity and energy

expenditure. However, literature displays similar achievements regardless of exercise order for muscle hypertrophy, while gains in muscular strength need to keep the size order in the session[241].

1.1.4.3 Rest Interval

The time taken to recovery between sets and exercises refers to the rest interval. Rest intervals can be classified into 3 categories: short (<60 s), moderate (60-120 s), and long (>120s min)[242]. The length of the rest interval depends on the training status, the training goal and the load lifted, constituting a primary determinant of the overall intensity[13].

Short rest intervals generate significant metabolic stress but cause a decreased total work capacity, limiting the hypertrophic benefits[243]. Long rest intervals allow a complete recovery of strength between sets but compromise the hypertrophic response[244] due to a reduced metabolic stress stimulus. Thus, moderate rest intervals appear to provide a satisfactory compromise between long and short rest periods for maximizing muscle growth[2].

Therefore, when prescribing training, literature largely supports rest intervals between 5-8 min for power, 3-5 min for maximal strength, 1-2 min for muscular hypertrophy and 30-60 s for muscular endurance[13].

1.1.4.4 Repetition Velocity

Repetition velocity is the time under tension of the muscle. The recommended repetition velocity is a 2:1:4 cadence (2s concentric action; 1s pause; 4s eccentric action)[245].

Some studies suggest that concentric actions at 1-s cadence are beneficial for hypertrophy due to an increased recruitment of high threshold motor units[246]. Other studies suggest that moderate velocities have greater effects on hypertrophy due to a heightened metabolic stress[247]. Moreover, when comparing slow velocity (2:1:4 cadence) with super slow velocity (10:1:5 cadence), the greater strength gains were associated with slow cadence[237]. However, for advanced trainers, the inclusion of slow, moderate and fast velocities may maximize strength and hypertrophy[21].

1.1.4.5 Muscular Failure

Muscular failure is the point during a set when muscles can no longer produce the necessary force to concentrically lift a given load[2]. This failure could be classified as momentary muscular failure or volitional failure (the maximum number of possible repetitions in a given set perceived by the athlete). Several theories hypothesize that training to muscular failure provides an additional stimulus for hypertrophy[248]. However, a recent meta-analysis determines that training to failure or not to failure produces similar increases in muscular strength and muscle size, although differences in training status or individuals' characteristics need future research[249].

1.2.—Hypoxia

Hypoxia is defined as any combination of reduced barometric pressure (PiB) and/or a reduced inspired fraction of oxygen (FiO_2), which ultimately results in an inspired partial pressure of oxygen (P_iO_2) less than 150 mmHg[250].

Over the past decade, training under hypoxic conditions has become a topic of great interest among elite athletes aiming to improve sea-level sports performance. Traditionally, since the 1968 Mexico Olympic Games, the ascent to higher altitudes has been associated with unexpected performance increments in explosive-related disciplines and impaired endurance performance[251,252]. However, when remaining at altitude, the influence of environmental hypoxia in the aerobic energy supply seems to elicit beneficial chronic adaptations which improve performance[253,254].

The exposure to hypoxic conditions triggers a respiratory, cardiovascular, muscular and nervous systems response, limiting performance and requiring an acclimatation process. The subsequent adaptations are determined at the molecular level by HIF-1α related processes[255], which are not activated to the same extent by training in normoxia or by passive hypoxic exposure[256]. HIF-1α regulates the transcription of genes related to erythropoiesis, angiogenesis, vasodilation, energy metabolism, apoptosis and catecholamine synthesis. There are also hypoxia-induced non-haematological changes, such as mitochondrial gene expression and enhanced muscle buffering capacity[257]. Combining haematological and non-haematological responses can result in increased maximal oxygen uptake (VO_2max) and/or competition performance at sea level[258].

For this reason, sojourns at terrestrial altitude have become popular among endurance athletes to improve their aerobic performance. Otherwise, the effect of altitude on R_T has received much less attention due to an association between chronic exposure and a deterioration in lean mass[259]. These different adaptations depend on the

hypoxic type (e. g., hypobaric vs. normobaric), severity (e.g., high altitude vs. moderate altitude) and training strategies (e.g., living and training at altitude vs. intermittent exposure). Thus, it is important to overview the protocol used when examining the training responses commonly used in elite sports.

Table 3 — The relationship between altitude (m) and barometric pressure (P_B), equivalent of the PiO_2 in percent at sea level (Eq PiO_2) and the equivalent of the FiO_2 at sea level (Eq FiO_2). Adapted from Leissner and Mahmood[262].

m	P_B	Eq PiO_2 at sea level	Eq F_iO_2 % at sea level
Sea level	760	100	20.9
1000	679	89	18.5
2000	605	79	16.4
3000	537	69	14.4
4000	475	60	12.6
5000	420	52	10.9
6000	369	46	9.5
7000	324	40	8.1
8000	284	34	6.9
9000	248	28	5.9
10000	215	24	4.9

1.2.1 Types

The hypoxia exposure typically used for research purposes can be achieved by the ascent to high altitudes (hypobaric hypoxia, HH; FiO_2 = 0.209; PB < 760 mmHg) or by breathing O_2-depleted air (normobaric hypoxia, NH; FiO_2 < 0.209; PB = 760 mmHg)[260]. Hypobaric hypoxia is generally considered from terrestrial altitudes higher than 1000 m[261]. Although the proportions of oxygen and nitrogen in the air are the same below 10.000 m of ascent, the progressive reduction in the air pressure leads to a decrease in the partial pressure of oxygen (PO_2) which makes it difficult for O_2 to diffuse into the lung capillaries

(Table 3)[262].On the other side normobaric hypoxia is produced by artificially reducing the FiO_2 (nitrogen dilution or oxygen filtration)[256]. The two main procedures to simulate hypoxia are the hypoxic chamber (altitude tents, rooms, etc.) and the face mask connected to a hypoxic air generator[253]. The reduction in FiO_2 by both methods induces systemic hypoxia, called hypoxemia, which in turn may reduce tissue oxygenation. During exercise, hypoxia can also occur, but contrary to the previous situations, the oxygen restriction is limited to the skeletal muscle involved[263].

Normobaric and hypobaric hypoxia have been shown to induce subtle different physiological adaptations, such as differences in ventilation[264], fluid balance[265], acute mountain sickness (AMS)[266], nitric oxide metabolism[267] and sport performance[268]. Thus, both types of hypoxia cannot be used interchangeably[266]. HH seems to represent a more severe physiological stimulus than NH at the same effective FiO_2[11]

1.2.2 Severity

Hypoxia classifications have been developed to determine the limits at which different physiological and stressors changes are observed, although the classification varies according to different authors. Hypoxia is commonly classified in four levels: low (1000-2000 m; FiO_2: 19.8-16.7%), moderate (2000-3000 m; FiO_2: 16.7-14.8%), high (3000-5500 m; FiO_2: 14.8-10.9%) and extreme (>5500 m; FiO_2: <10.9%)[269]. The specific physiological and physical responses depend on the combining effect of exposure duration and hypoxia severity[256,270].

The exposure to a severe hypoxia (altitudes ≥ 5000 m) has been associated to mountaineering or alpinism. There is a general agreement that exposure to real or simulated altitude over the periods of Himalayan expeditions (5-6 weeks) leads to a 10% to 15% loss of muscle volume[271] and a concomitant 20% to 25% decrease in muscle fiber size[272] due to a decrease in muscle protein synthesis[273]. However, training camps are usually performed at moderate altitude[256,274], which is considered an optimal level for training purposes[268,275].

At moderate altitude, athletes seem to benefit from the hypoxic stimulus, with subsequent performance improvements at sea level, while the main

disadvantages associated, such as AMS symptoms, are likely minimized[276]. Ascent and permanent exposure to higher altitudes can induce health alterations (edema, pulmonary hypertension, etc.), for which an acclimation process is advisable over 2500 m[276,277]. There are several altitude training centers over the world ranging between 1200 and 3600 m[256]. One of them is located in Granada, the High-Performance Center of Sierra Nevada, at 2320m asl. (Figure 7). Moreover, the artificial hypoxic devices allow combining hypoxic methods (terrestrial and simulated hypoxia) to elicit different targets (aerobic, anaerobic, muscular, etc.) along the periodization process[274,278] at the same time they can be used just before, during and/or after an altitude training camp[274] .

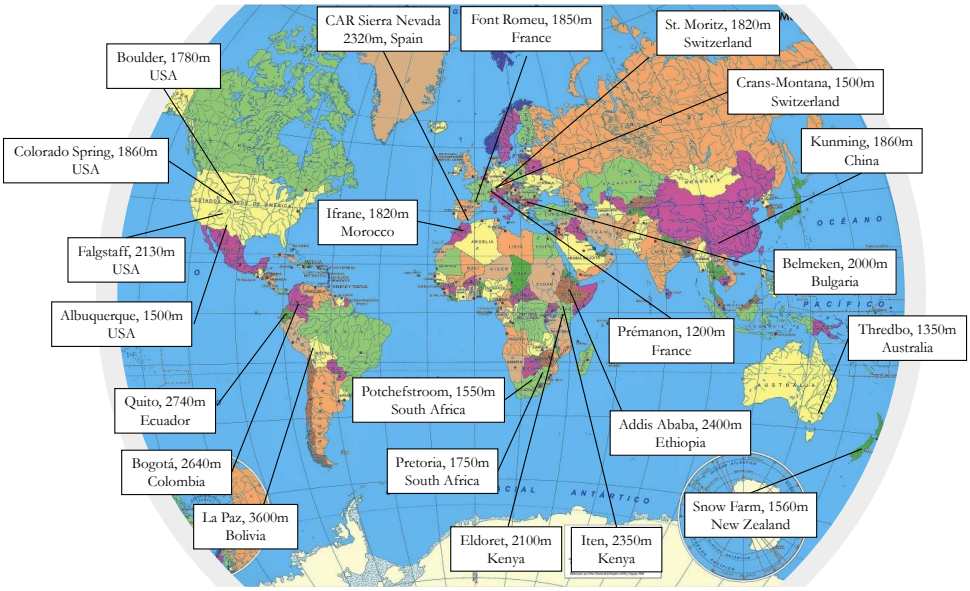


Figure 7 — Altitude training places around the world. Adapted from Fisiología del Ejercicio (p. 353) by Feriche, B [263].

1.2.3 Hypoxic training strategies

The combination between hypoxic types (i.e., hypobaric or normobaric), and severity of the hypoxia during the training and living periods encourage the development of multiple training strategies (Figure 8) aiming to enhance performance or acclimatation to perform at altitude.

The most often hypoxic strategy adopted by athletes is living and training for several weeks at terrestrial moderate altitude in the lead up to competition, termed "living high-training high" (LHTH)[256]. Altitude training has grown in popularity but traveling and sojourn in a mountain center is not always feasible and is costly and logistically difficult for a large number of athletes.

The traditional LHTH altitude training strategy was replaced or complemented by the "live high-train low" (LHTL) strategy[280]. The LHTL allows to combine the beneficial effects of a prolonged exposure to hypoxia (>12h/day) with the maintenance of high intensity trainings whilst avoiding the detrimental effects of chronic hypoxia (such as muscular mass loss, fatigue or deteriorated aerobic performance[281]). Levine's research team first investigated the LHTL method by transporting the athletes from sea level or low altitude (<1300 m) to train whilst spending the rest of their time living and sleeping at moderate altitude (1800–2500 m)[282]. However, this method caused a large amount of stress to the athletes as a result of travelling to and from training sites, adapting to differences between altitude and sea level or financial costs. For this reason, the development of devices to simulate altitude conditions for longer periods (i.e., hypoxic sleeping units or altitude tents) made it possible to use artificial altitude without travelling to the mountains[256].

Another way to benefit from hypoxic stimulus without the detrimental effects of a prolonged exposure to hypoxia is to train under hypoxic conditions and to remain at sea level for the rest of the time[278]. "Live low-train high" (LLTH) strategy or intermittent hypoxic training consist in an exposure to hypoxia (typically last <2 h at rest or while training 2-5 times per week), that only causes a slight disturbance to athletes' usual daily routine[254], and preserve the sleep quality or recovery. Many studies have reported information about the hematological and muscular levels and their consequences on performance. However, the benefits may vary across individuals, in particular when the focus of their training also differs (aerobic

or anaerobic adaptations). This has motivated the technological development of new devices as tents, hypobaric chambers, hypoxic masks and hypoxic rooms[278] and renewed the interest in LLTH interventions using either local (BFR) or systemic hypoxia strategies (interval hypoxic training (IHT), repeated-sprint training in hypoxia (RSH) or RTH, among others) to elicit metabolic and neural adaptations.

Therefore, the improvement of hypoxic training strategies that have been traditionally focused on the enhancement of endurance are now extended to RTH[7–10] and RSH as a result of the potential benefits favoring the hypoxic environment[283–285]. Nevertheless, the emerging literature evidence about the potential benefits of hypoxia should be carefully considered when examining the training adaptative response in terms of hypoxic type, severity and training strategy.

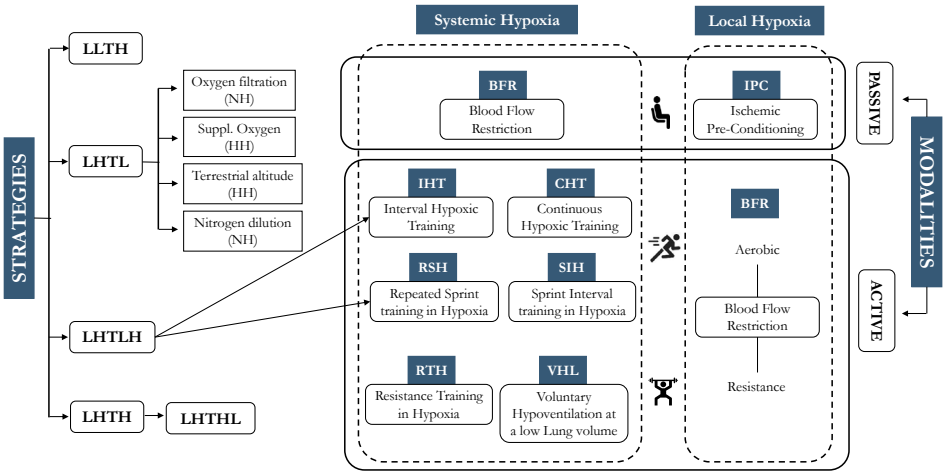


Figure 8 — Hypoxic training strategies. Adapted from Girard et al,[278,284]. LHTH, 'live high-train-high'; LHTHL, 'live high-train high and low'; LHTL, "live high-train low'; LHTLH, 'live high-train low and high'; LLTH, 'live low-train high'; IHE, intermittent hypoxic exposure; CHT, continuous hypoxic training; IHT, intermittent hypoxic interval training; RSH, repeated sprint training in hypoxia; RTH, resistance training in hypoxia; IHIT, IHE + IHT; NH, normobaric hypoxia; HH, hypobaric hypoxia.

1.3.—Resistance training under hypoxic conditions

The effects of terrestrial hypoxia on acutThe effects of natural hypoxia on acute and chronic responses to strength training remain inconclusive and limited to high altitudes exposures or simulated conditions. Exposure to higher altitudes has been presented as a harmful environment for skeletal muscle development[286] due to a negative balance depicted between muscle protein synthesis and degradation[287]. However, higher altitudes differ from the ones commonly used for sport training camps (2000-3000 m) where the availability of oxygen seems not to affect the protein synthesis rate[288], and nutrition, sleep and training conditions are carefully controlled[289].

The use of the moderate hypoxia has been progressively introduced to maximize muscle strength adaptations[8,259]. R_T carried out under intermittent hypoxic conditions seems to maximize muscle adaptations[290] and it could be used to target other training goals that require longer adaptation periods (i.e., strength training related). The recent narrative reviews[7,8,10] and meta-analysis[9] concluded that RTH did not support a clear added benefit for muscle mass and strength compared to the same training in nomoxia. However, discrepancies among training protocols (populations, training protocols, muscles involved, hypoxic dose) do not allow a good comparison between studies and draw firm conclusions. Moreover, it is expected that terrestrial altitude produces different and more severe physiological adaptations than simulated conditions[11,260], highlining the necessity to assess this gap.

1.3.1 Acute response to RTH

Skeletal muscle mass is regulated by the balance between protein synthesis/degradation[291] and myogenesis (satellite cell activation, proliferation, differentiation and fusion (Figure 9))[37].

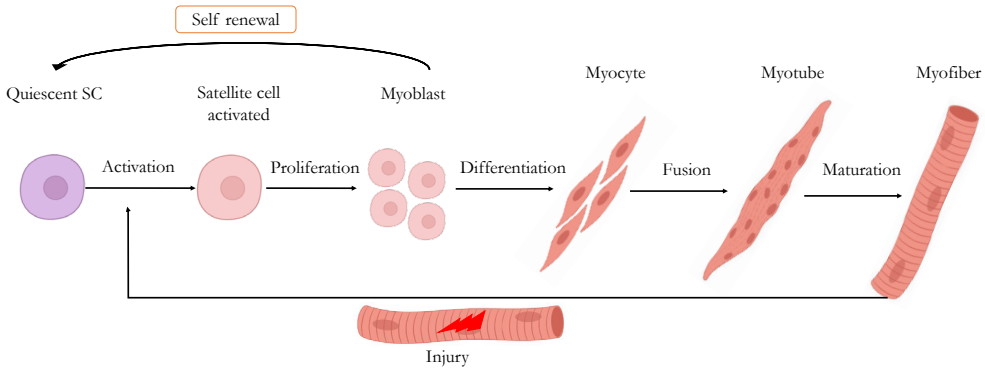


Figure 9. Skeletal muscle regeneration process.

Exercise under hypoxic conditions is characterized by a greater muscle oxygen desaturation, an increased anaerobic metabolism, and therefore a potential increase in the production of metabolites (such as lactate, H^+ and Pi[64,67,69,290,292]) and changes in pH levels[293]. All these apparently greater responses could potentiate the myogenic activation, translated into satellite cell incorporation in the long-term[294]. A higher metabolic stress also seems to promote the recruitment of high-threshold motor units[64,290] and supports a benefit of training to enhance the hypertrophic response due to the adaptation of increased muscle fibers to the training stress[293]. However, when examining the acute response of hypoxia after a R_T session, the literature displays hormonal and metabolic inconsistent results.

Particularly, greater increments in blood lactate[68,71,295], serum GH[67–69], serum cortisol[69] and a decrease in HCO_3[71] have been reported after R_T exercise at acute moderate and severe hypoxia compared to normoxia conditions. Contrary, other studies have failed to detect differences in post-exercise testosterone[67,69], serum IGF-1[69] and blood lactate[69,71,296,297] between N and H conditions, although they remained higher than pre-exercise. Moreover, it is also expected that the hypoxic condition improves the hypertrophic response after a resistance exercise (i.e., 8 sets x 8 reps at 80% 1RM, 14% FiO_2) by the myogenic activation and the inflammatory response through IL-6/STAT3-dependent myogenesis and immune cells-dependent muscle regeneration[226].

Some hypotheses could explain the differences among the available results. For example, circulating IGF-1 may not be altered by exercise in both conditions or even the production could be autocrine and therefore undetectable in blood[226]. It is also possible that molecules such as lactate need more time under tension[297] or more muscle groups recruited[296]. Even it is possible that differences among the protocols used (sets, repetitions, rest, hypoxia severity, exercises, etc.) could affect the metabolic and hormone response[8].

Therefore, although an increased metabolic stress may occur, more experiments need to be performed to identify the role of hormones, metabolites, or signaling pathways related to hypertrophy among others when training under acute or chronic hypoxic conditions.

1.3.2 Chronic responses to RTH

Based on the acute effects of hypoxia-induced increases in metabolic stress linked to the lack of oxygen availability, and on the effect it has on RTH-induced hypertrophy[64,67,69,292,293], RTH could promote greater improvements in muscle size and strength than the same training in normoxia[67,71,298]. Moreover, it is suggested that these improvements could be achieved in a shorter time under hypoxic conditions[299,300].

Previous systematic reviews[7–10] did not report substantial differences compared to training in normoxia and have highlighted discrepancies among training protocols when comparing both methods. The main discrepancies found include: different training loads (from 30 to 90% 1RM), inter-set rest intervals (from 30 to 180 s), number of sets (from 3 to 6), training period lengths (from 3 to 8 weeks), severity of hypoxia (from 12 to 16% of FiO_2), participant training levels (untrained, recreational trained, recreational resistance trained, strength trained, well-trained in a sport discipline and professional athletes) or session type and muscles worked on (isolated small or big muscles vs. full-body sessions) which makes difficult to draw firm conclusions on the potential benefit of RTH vs. RTN.

In particular, the meta-analysis conducted by Ramos-Campo et al.,[9] compared the effects of RTH vs. RTN on muscle CSA, lean mass and strength without finding an additional benefit

in favor of the hypoxia condition (standardized mean differences (SMD)=0.24 [-0.19; 0.68] and 0.20 [-0.13; 0.53] for CSA and 1RM, respectively). Although the authors established different subgroups in order to assess the possible heterogeneity biases (upper and lower limbs), they could not find an added benefit of RTH on CSA and 1RM and were unable to assess any other methodological discrepancy due to the reduced number of available research to that date.

Since 2018, several studies on the topic have been published, thereby providing an opportunity to achieve greater statistical power when meta-analyzing data on the effects of RTH. The recent narrative review by Deldicque[7] includes 16 studies and extends the discussion of RTH to performance-based outcomes such as velocity and muscular power. However, given its narrative format, this review did not seek to quantify the effect of RTH on muscular adaptations and its non-systematic approach introduces the potential for selection bias[301], thereby limiting the veracity of its conclusions.

To address the gaps in the current literature, it could be interesting to analyse the potential impact of methodological covariates, such as training status, the training load, inter-set rest interval, and the severity and type of hypoxia used, among others. Future investigations on altitude training as a performance enhancing strategy for elite endurance athletes should determine the long-term effects of accumulated altitude training through repeated exposures, as well as the interactions between altitude and other components of a periodized approach to athletic preparation at altitude, such as recovery, nutrition, and psychological skills training

1.3.3 Differences between HH and NH

Although the physiological and performance responses to NH versus HH do appear minor when accounting for equivalent hypoxic dose[12], there are subtle differences. This "equivalent air altitude model" has now been criticized and a growing body of literature has reported physiological differences between acute exposures to NH and HH[302]. Specifically, exposures to HH need a pre-acclimatation process in order to reduce the AMS, whose

symptoms are less severe under NH[303]. However, the ascending to altitude entails an acute acclimatization process that does not occur in NH, where the time spent between placing the device or entering the room and the start of the exercise is reduced to 5-10 minutes of exposure. Moreover, it is not clear whether the same effects would be seen with or without an acclimatation process between conditions.

Furthermore, NH and HH could not "be used interchangeably" with HH representing a more severe physiological stimulus than NH at the same effective FiO_2[260]. The main difference between both types of hypoxia is the PB, that could explain the different physiological adaptations caused by differences in ventilation, fluid balance, AMS, nitric oxide metabolism, and sport performance. This would also make the assumption that larger physiological adaptations would occur after prolonged hypoxic exposure under HH compared to NH conditions. However, this hypothesis still needs to be demonstrated in research.

Finally, the use of NH in R_T could be beneficial to develop muscle hypertrophy. At least, 8 weeks are necessary to observe changes in CSA, which limits its application in HH. The benefit of hypoxia in hypertrophy training is based on the potential intensification of the metabolic stress response and the mechanisms related to muscle growth. But, as mentioned, the different physiological responses between HH and NH should not exclude HH as a more efficient metabolic stress stimulus to R_T. However, the presence of confounding factors frequently not addressed in the studies, such as acclimatization time spent in hypoxia, temperature, carbonic accumulation and humidity, and the limited statistical power due to small sample sizes, limits the conclusions that can be drawn from these findings[304].

Nonetheless, it should be noted that strength training under hypoxic conditions is still a topic of great interest due to the inconclusive results reported and the lack of the studies conducted under terrestrial hypoxia. Thus, it could be important to improve the quality and quantity of future studies in hypoxia to allow the comparability of results.

1.4.—Statement of the problem

The literature reviewed highlights the need for hypoxic training as a new strategy to increase the stimulus and outcomes in addition to traditional training. The effect of the hypoxic stimulus has been traditionally studied to improve aerobic performance at terrestrial altitude due to the physiological benefits associated with performance. However, the development of new devices to simulate the hypoxic stimulus has increased the interest in RTH and open a new area of research because of the potential different physiological responses between terrestrial and simulated hypoxia. Thus, controlling variables such as the type of hypoxia (simulated vs. terrestrial), the exposure time (acute, chronic, or intermittent) or the training methodology (load, sets, volume, exercises, etc.) has become an area of research to determine the role of the hypoxia on the success of a R_T period with respect to normoxia.

Additionally, no research has investigated the physiological and performance-related responses of altitude after a R_T period. The last systematic reviews suggest the use of hypertrophy R_T protocols (moderate loads and intensity and short inter-set rest intervals) combined with a moderate severity of the hypoxia to maximize the muscle adaptations. Nevertheless, the increased number of studies on the topic published in the last years make it necessary to address the gaps in the current literature and sub-analyze the potential impact of methodological covariates on the results, such as the training load, inter-set rest interval, and the severity of hypoxia.

Although the literature considers metabolic stress and the subsequent metabolic pathways activation (IGF1/PI3K/AKT/mTOR) essential to muscle hypertrophy, the physiological adaptations are further for a complete understanding. However, the relative incipient discovery of new molecules involved in these processes (such as cytokines or miRNAs) could elucidate the biomolecular responses and their relevance for skeletal muscle adaptations to R_T as well as the impact of the hypoxia on these results (RTH). Moreover, the literature suggests differences between HH and NH, although any study has compared both types of hypoxia on muscle mass and strength

development. Therefore, comprehensive comparative research on R_T carried out in both terrestrial and simulated hypoxia will allow to better understand the hypertrophy and strength adaptations and sets the main objective of this thesis.

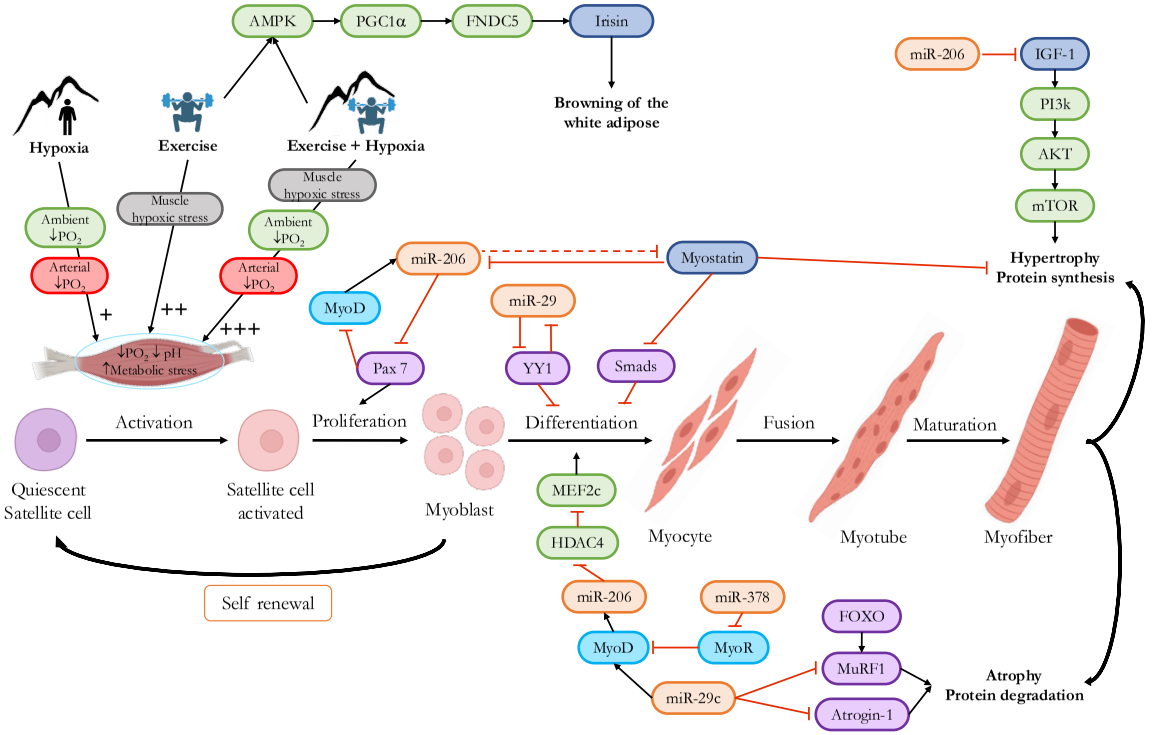


Figure 10 — Overview and contribution of hypoxia and exercise in the myogenesis process. AMPK: 5' adenosine monophosphate-activated protein kinase ; PGC1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; FNDC5: fibronectin type III domain-containing protein 5; PO2: ; IGF-1: insulin-like growth factor 1; PI3k: phosphatidylinositol 3-kinase; AKT: protein kinase B; mTOR: mammalian target of rapamycin; miR: microRNA; myoD: myoblast determination protein 1; Pax 7: paired box protein Pax-7; YY1: Yin Yang 1; MEF2c: myocyte-specific enhancer factor 2C; HDAC4: histone deacetylase 4; FOXO: forkhead box O; MuRF1: muscle ring finger-1. → Activation, ⊣ inhibition, ⊣ inhibition in Texel sheep.

2

Aims and hypothesis

2.1. General aim

The main aim of this doctoral thesis was:

To analyze the effect of the resistance training exercise under acute and chronic moderate hypoxia conditions of different types (terrestrial vs. simulated) on the physiological response and muscle and strength performance markers.

2.2. Specific aims

Specific objectives and hypotheses have been targeted in four different sections:

Section I. Systematic review and meta-analysis.

Study 1. To perform an updated systematic review and meta-analysis to explore the effect of RTH on muscle hypertrophy and strength development and to sub-analyze the potential impact of methodological covariates, such as the training load, inter-set rest interval, and the severity of hypoxia. The analysis thus will enhance the ability to provide specific recommendations about the effectiveness of RTH, as well as to detect procedural gaps in the literature that spurs future research on the topic.

Section II. Preliminary studies on acute exposure to hypobaric hypoxia.

Study 2. . To compare the acute response of a hypertrophy-oriented R_T session under normoxic conditions and after the ascent to a moderate altitude on exercise-induced stress variables associated with muscular gains (metabolites, ions and anabolic hormones). We hypothesized that the exposure to altitude would produce an increased stress response compared to the normoxia condition and thus enhance acute factors associated with muscle hypertrophy.

Study 3. To compare the acute effects of a hypertrophy-oriented R_T session under normoxia and terrestrial moderate hypoxia on exercise-induced inflammatory cytokines and anabolic hormones associated with muscular adaptations; and to establish the influence of acute altitude on the potential relationship between miR-378 and these related variables. We hypothesized that the acute hypertrophy-oriented R_T session would increase the inflammatory, hormonal and miR-378 responses, and that hypoxia would amplify these results.

Table 4 — Characteristics of the different studies carried out in the present Doctoral Thesis.

	Study	Title	Experimental design	Condition	Hypoxic exposure	n	Methods	Statistical methods
Section I	1	Efficacy of resistance training in hypoxia on muscle hypertrophy and strength development: a systematic review with meta-analysis. ***Benavente et al, 2023***	Intergroup (N vs. NH) Pre-post	Systematic review and meta-analysis	NH	17 studies	Resistance training	Meta-analysis
Section II	2	Altitude-induced effects on muscular metabolic stress and hypertrophy-related factors after a resistance training session. ***Feriche et al, 2020.***	Intragroup (N vs HH)	Acute	HH	13	Full-body: 6 exercises 3sets x 10RM x 2 min rest	ANOVA Standardized mean differences Correlational analysis
	3	Hormonal and Inflammatory Responses to Hypertrophy-Oriented Resistance Training at Acute Moderate Altitude. **Benavente et al*, 2021.***						
Section III	4	Inter-set rest configuration effect on acute physiological and performance-related responses to a resistance training session in terrestrial vs simulated hypoxia. ***Benavente et al, 2022.***	2 groups: Intergroup (HH vs. NH) and intragroup (N vs. H) 1 vs 2 min	Acute	G1 HH G2 NH	G1:9 G2:7	Full-body: 6 exercises 3 sets x10 reps. (12RM) x 1- or 2-min rest	Mixed-effect model Standardized mean differences
	5	Effects of resistance training in hypobaric vs normobaric hypoxia on circulating ions and hormones. ***Timón et al, 2022.***						ANOVA Standardized mean differences
	6	Influence of the terrestrial vs simulated hypoxia on biomolecular anabolic signalling response to resistance exercise: A pilot study. ***Feriche et al, 2023*** **[Submitted].**					Full-body: 6 exercises 3 sets x10 reps. (12RM) x 1 min rest	Mixed-effect model Standardized mean differences
Section IV	7	Strength and muscle mass development after a resistance-training period at terrestrial and simulated intermittent hypoxia. ***Benavente et al., 2023*** **[Under writing process].**	3 groups Intergroup (N vs. HH vs. NH)	Chronic	N HH NH	10 10 13	Full-body: 6 exercises 3 sets x 8-12 reps. (65-80% 1RM) x 90 s rest	ANOVA Kruskal Wallis Wilcoxon Standardized mean differences

N: normoxia; NH: normobaric hypoxia; HH: hypobaric hypoxia; RM: repetition maximum; reps: repetitions.

Section III. Acute effect and comparison between types of hypoxia.

Study 4. To compare the effect of different types of acute hypoxia (HH vs. NH) combined with different inter-set rest configurations (60 s vs. 120 s) during a traditional hypertrophy-oriented R_T session on perceptual, physiological and muscle performance markers. We hypothesized that short rest periods would produce higher perceptual, cardiovascular and blood lactate changes, and its combination with terrestrial hypoxia would maximize this response.

Study 5. To analyze the effect of the hypoxic environment on hormonal and circulating ions responses after performing high-intensity R_T with different inter-set rest under HH and NH. We hypothesized that high-intensity resistance training with short inter-set rest under HH conditions produces a greater hormonal and metabolic responses than under NH, since the reliance on anaerobic metabolism is increased.

Study 6. To compare the acute myokine/miRNA response to a traditional hypertrophy session under two different but equivalent types of moderate hypoxia (terrestrial vs. simulated). We hypothesized that the hypoxia would produce a fast increase in myokine and miRNA expression compared to sea level, and enhance acute mediators associated with muscle hypertrophy. The type of hypoxia will affect the magnitude of the response.

Section IV. Chronic effect and comparison between types of hypoxia.

Study 7. To investigate the effect of an 8-week R_T period under terrestrial and simulated hypoxia on muscle mass and strength development with respect to the same training in normoxia. We hypothesize that the training period in hypoxia will increase muscle hypertrophy and maximal strength more than in normoxia. These changes will attend to the metabolic stress and corresponded myogenesis marker response.

3

Methods

Studies' methodological overview

The present Doctoral Thesis contains a total of 7 studies. They are classified into four different sections depending on the study aim and the methodology used.

All the studies contain data from healthy resistance-trained males, although the training status analyzed from the systematic review ranged between untrained to well-trained participants. The data collection in the current Doctoral Thesis was completed through training session monitoring (load, repetitions, inter-set rest intervals, etc.) blood measurements (hormones, metabolites, cytokines, genetic markers, etc.) and perceptual and cardiovascular responses (RPE, HR, etc.). The measurements within each study were taken acutely or chronically depending on the aim of the study to compare the effect of resistance training on hypoxia as well as the difference between types of hypoxia.

Each study was carried out following the current Spanish legal regulations that control human research. Moreover, each study was approved by the corresponding Research Ethics Committee in accordance with the Helsinki Declaration. All participants were provided with information detailing the purpose and requirements of the research protocol of each study and provided signed informed consent.

An overview of the design, participants, variables and statistical analysis included in all studies is displayed in Table 5. After it, the method of each study is presented separately by sections, to better understand the followed procedure.

Table 5 — Studies' methodological overview.

	Study	Experimental design	Participants	Effective FiO_2	Training level	Age (years)	Weight (kg)	Methods	Variables studied	Statistical methods
Section I	1	Systematic review and meta-analysis Intergroup (N vs NH) Pre-post	17 studies	N: 21% FiO_2 NH: 12-16% FiO_2 (~4500 to 2300 m asl.)	Untrained Recreational Trained in R_T Well-trained	20.2 ± 3.3 to 29.0 ± 5.0	60.2 ± 16 to 98.7 ± 12.8	Resistance training	1RM CSA Muscle thickness Lean mass	Meta-analysis
Section II	2 3	Acute Intragroup (N vs HH)	13 males	N: <700 m asl. (21% FiO_2) HH: 2320 m asl (15.9% FiO_2)	Trained in R_T	22.31 ± 2.59	76.92 ± 9.17	Full-body: 6 exercises 3 sets x 10RM x 2 min rest	Volume-load (BS, BP, MP) Lac, Pi, CO_2L, Ca^{2+} T, GH, C IL-6, 10, TNFα, miR-378	ANOVA Standardized mean differences Correlational analysis
Section III	4 5 6	Acute 2 groups: Intergroup (HH vs NH) and intragroup (N vs H) 1 vs 2 min	N/HH: 9 males N/NH: 7 males	N: <700 m asl. (21% FiO_2) HH: 2320 m asl. (15.9% FiO_2) NH: 15.9% FiO_2 (~2320 m asl.)	Trained in R_T	23.6 ± 3.2 26.0 ± 3.0	73.9 ± 5.3 73.9 ± 7.8	Full-body: 6 exercises 3 sets x 10 reps. (12RM) x 1- or 2-min rest Full-body: 6 exercises 3 sets x 10 reps. (12RM) x 1 min rest	Volume-load (BS and BP) maxLac, HR, RPE, SmO_2 Ca^{2+}, HCO_3^-, Pi, GH, C, Lac Irisin, Myostatin, miR-378, miR-206, miR-29c	Mixed-effect model Standardized mean differences ANOVA Standardized mean differences Mixed-effect model Standardized mean differences
Section IV	7	Chronic 3 groups Intergroup (N vs. HH vs. NH)	N: 10 males HH: 10 males NH: 13 males	N: <700 m asl. (21% FiO_2) HH: 2320 m asl. (15.9% FiO_2) NH: 15.9% FiO_2 (~2320 m asl.)	Trained in R_T	22.7 ± 3.4 22.8 ± 4.2 21.9 ± 2.2	72.0 ± 7.2 74.0 ± 13.9 75.0 ± 8.9	Full-body: 6 exercises 3 sets x 6-12 reps. (8-15RM) x 90 s rest	1RM, Muscle thickness GH, IGF-1, % active mTOR, lactate, IL-6, TNFα, miR-206, miR-378, miR-29c	ANOVA Kruskal Wallis Wilcoxon Standardized mean differences

N: normoxia; NH: normobaric hypoxia; HH: hypobaric hypoxia; FiO_2: inspired fraction of oxygen; asl: above sea level; Trained in R_T: participants who achieved at least 12 months of continuous resistance training history immediately prior to the study; RM: repetition maximum; reps: repetitions; CSA: cross-sectional area; BS: back squat; BP: bench press; MP: military press; Lac: lactate; Pi: inorganic phosphate; CO_2-L: liquid carbon dioxide; Ca^{2+}: calcium; GH: growth hormone; C: cortisol; T: testosterone; IL: interleukin; TNFα: tumor necrosis factor α; % active mTOR: % of active mammalian target of rapamycin (% active mTOR = active mTOR/total mTOR x 100); miR: microRNA; SmO_2: Muscle oxygen saturation; HCO_3^-: bicarbonate; IGF-1: insulin-like growth factor 1.

Section I. Systematic review and meta-analysis.

Study 1. Efficacy of resistance training in hypoxia on muscle hypertrophy and strength development: a systematic review with meta-analysis.

Benavente, C., Schoenfeld, B. J., Padial, P., & Feriche, B. (2023). Efficacy of resistance training in hypoxia on muscle hypertrophy and strength development: a systematic review with meta-analysis. Scientific Reports, 13(1), 3676.

» Study Design

This meta-analysis followed the recommendations described in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[305] (see Tables S1 and S2).

» Data sources and search strategy

A systematic search was performed using PubMed-Medline, Web of Science, Sport Discus and the Cochrane Library from database inception through 7 June 2022. The following combination of terms was used for the search: ("strength training" OR "resistance training" OR "weight training") AND ("hypoxia" OR "altitude" OR "hypoxic training") (see Table S3 for specific search strategies), without a restriction of date of publication. The search was performed individually by two authors. Full texts of studies deemed potentially relevant based on title and abstract were screened, and a final decision was then made as to whether a study warranted inclusion. Any discrepancies were resolved through discussion; if needed, a third author arbitrated to arrive at a final decision.

» Inclusion criteria

We included studies that (1) examined the effect of R_T under intermittent terrestrial or simulated hypoxia for at least 3 weeks [56] on muscle hypertrophy (CSA, lean mass or muscle thickness) and strength development (1RM) in healthy individuals between 18 and 65 years of age using a randomized design; (2) included a normoxic control group; (3) were published in English-language peer-reviewed journals; and (4) provided information about outcomes both at baseline and post-study. Research studies were excluded if they (1) were not original investigations published in full; (2) did not specify the tests to be evaluated; (3) applied hypoxia via local techniques, such as blood flow

restriction; (4) did not provide or specify numerical or graphic data; and (5) examined only the acute effects of interventions.

» Data extraction and study outcomes

Data extraction of the included studies was conducted in a standardised manner by two authors independently. To ensure the reliability of this process, each author performed the data extraction of all studies included in the meta-analyses and separately entered the data into a spreadsheet. The data were then crosschecked and combined into a single spreadsheet for analysis.

For each included study we extracted the following data: authors and year of publication; sample size and mean age and weight of participants for each group; the type of hypoxic environment; the FiO_2; and the training status (in the absence of a specific description of participant training status, we defaulted to the description provided by the authors). The information extracted about the characteristics of the R_T programs included: training frequency (sessions/week), relative load lifted, sets, proximity to failure, inter-set rest interval, the type of exercise and the outcomes measured (i.e., maximal strength, CSA and/or lean mass and/or muscle thickness) (Table 6). In cases where data were not sufficiently reported, we contacted the authors of the relevant studies for additional information. In cases where an article presented results using figures, two authors extracted the values of the outcomes using online software (WebPlotDigitizer)[306]. When disagreement reached 3%, a third investigator extracted the data with the online software. The mean of the two closest derived assessments was used for analysis.

To assess potential confounding from covariates, we carried out subanalyses of data on the effects of training load (low < 50% 1RM); moderate = 60-80% 1RM; heavy >80% 1RM), inter-set rest interval (short ≤ 60s; moderate > 60 to < 120s; long ≥120s) and severity of hypoxia (moderate = 14.3 to 16 % FiO_2; severe <14.3 to 11% FiO_2).

» Evaluation of the methodology of the studies selected.

All trials included in the meta-analysis were assessed for methodological quality using relevant items from the Cochrane's risk of bias tool[307]

and the PEDro scale[308] by two with a third investigator in case of disagreement. The assessment of the selected studies included specification of eligibility criteria, random sequence generation, allocation concealment, inter-group similarity in main study outcomes at baseline, blinding of participants, blinding of outcome, incomplete outcome data and selective reporting (Table S4).

» Statistical analysis

All studies were independently analysed for each main outcome (CSA, lean mass and 1RM) using pre- and post-study results under hypoxic or normoxic conditions with values expressed as standardised mean differences (SMD) and their 90% confidence intervals (CIs). Subgroup analyses were performed to determine possible confounding effects of load, inter-set rest interval, and severity of the hypoxia. Standardised effect size coefficients from RCTs were computed as the mean differences between the mean change in hypoxic and normoxic groups from baseline to post-intervention, divided by the mean pooled baseline standard deviation[309]: In the intra-group pre-post measurement, the mean change from baseline to post-intervention, divided by the pooled baseline standard deviation, was used to calculate the standardised effect size coefficient for each intervention group: . Both coefficients included correction factors for small samples and [310]. The inverse variance method was used in both cases for the weighting of studies (Table S5). Additionally, we calculated the raw (unstandardised) mean difference for pre-post studies and RCTs by using the weights from our standardised meta-analysis to estimate the pooled mean difference in each outcome.

We analysed data using a multi-level random effects model to account for multiple effects nested within groups, studies and participants (three-level analysis)[311]. This approach allows to control for the bias of combining several measures from the same study (Figure S1). Independent effect size coefficients from studies and outcomes were combined and analysed using the DerSimonian and Laird's[312] random effects model. The weighted standardised mean change from baseline to post-intervention was the pooled effect size of each outcome.

Table 6 — Main Characteristics of included studies in the meta-analysis

Study	n	H condition	Effective FiO_2	Training level	Age (years)	Weight (Kg)	Training intervention			Variables measured	
							Weeks (s/w)	Methodology	Exercise	Strength development	Muscle hypertrophy
Chycki (2016)	6 (M) 6 (M)	NH (chamber) N (chamber)	12.9% FiO_2 21% FiO_2	Rec. Resistance trained	21±2.4 22±1.5	80.6±12.3 81.1±7.5	6 (2)	8 sets x 10 reps. 70%RM (Rest 180 s)	Bench press Barbell Squat		Lean Mass
Fashi (2021)	7 (M) 7 (M)	NH (gas) N (gas)	12,7% FiO_2 20,9% FiO_2	Untrained	21±4		4 (3)	3 sets x reps. to failure. 50% 10RM (~37% RM) (Rest 60 s)	Back squat	RM	CSA
Friedman (2003)	10 (M) 9 (M)	NH (room) N (room)	12% FiO_2 21% FiO_2	Untrained and recreational	25.1±2.9 24.3±2.5	77.0±9.0 72.9±9.0	4 (3)	6 sets x 25 reps. 30%RM (Rest 60 s)	Knee extension		CSA
Ho (2014)	9 (M) 9 (M)	NH (chamber) N (chamber)	15% FiO_2 21% FiO_2	Rec. trained	21.4±2.2 21.2±1.9	66.5±8.2 67.9±9.5	6 (3)	3 sets x 10 RM (75% RM) (Rest 120 s)	Squat	RM	Lean mass
Honda (2020)	9 (M) 7 (M)	NH (room) N (room)	14.4% FiO_2 21% FiO_2	Untrained and recreational	29±5 29±4	68.2±6.7 65.8±9.7	8 (2)	5 sets x 10 reps. 70%RM (Rest 90 s)	Bench press Leg press	RM	Lean mass
Inness (2016)	10 (M) 10 (M)	NH (facemask) N (facemask)	14.3% FiO_2 20% FiO_2	Strength trained	Bt 18-34	83.1±7.5 80.2±12.0	7 (3)	2-4 sets x 3-6 reps. 75%RM (Rest 180 s)	Squat Deadlift Lunge	RM	
Kon (2014)	9 (M) 7 (M)	NH (room) N (room)	14.4% FiO_2 21% FiO_2	Rec. resistance trained	28.4±1.6 28.2±1.4	68.2±2.2 65.8±3.7	8 (2)	5 sets x 10 reps. 70% RM (Rest 90 s)	Bench press Leg press	RM	CSA Lean mass
Kurobe (2015)	6 (M) 7 (M)	NH (room) N (room)	12.7% FiO_2 20.9% FiO_2	Untrained	23.0±1.0	60.2±1.6	8 (3)	3 sets x reps. to failure. 10RM (75% RM) (Rest 60 s)	Elbow extensions	RM	Muscle thickness
Manimmanakorn (2013)	10 (F) 10 (F)	NH (facemask) N (facemask)	80% SpO_2 21% FiO_2	Well-trained netball players	20.2±3.3	65.2±6.5	5 (3)	3 sets x reps. to failure. 20%RM (Rest 30s bt. set and 120 s bt. exercises)	Knee flexion Knee extension		CSA
Martínez-Guardado (2019)	15 (M) 13 (M)	NH (chamber) N (chamber)	15% FiO_2 20.9% FiO_2	Strength trained	24.6±6.8 23.2±5.2	74.9±11.5 69.4±7.4	8 (2)	3 rounds x 2 blocks x 3x6RM (85%RM) (Rest 35s bt exercises, 180s bt sets, 5 min bt blocks)	Bench press Leg extension Front pull down Deadlift Preacher curl Calf raises	RM	Lean mass
Martínez-Guardado (2020)	16 (M) 16 (M)	NH (chamber) N (chamber)	13% FiO_2 21% FiO_2	Untrained	25.7±6.4	74.7±12.9 81.1±11.7	7 (3)	3 sets x reps. to failure. 65-75-80%RM (Rest 90s)	Bench press Bicep's curl French press Pendlay row Half squat	RM	Lean mass

Table 6 — Continued...

Study	n	H condition	Effective FiO_2	Training level	Age (years)	Weight (Kg)	Training intervention			Variables measured	
							Weeks (s/w)	Methodology	Exercise	Strength development	Muscle hypertrophy
Mayo (2018)	8 (M) 9 (M)	NH (chamber) N (chamber)	14.4% FiO_2 20.9% FiO_2	Professional rugby athletes	24±3	98.7±12.8	3 (4)	1-12 sets x 2-4 reps. 85-92.5%RM (Rest 180 s bt. super sets)	Back squat Bench press Weighted Chin-up	RM	
Nishimura (2010)	7 (M) 7 (M)	NH (room) N (outside)	16% FiO_2 21% FiO_2	Untrained	22.7±2.7 21.6±1.6	66.8±6.0 65.0±8.1	6 (2)	4 sets x 10 reps. 70% RM (Rest 60 s)	Elbow flexion Elbow extension	RM	CSA flexors CSA extensors
Ramos-Campo (2019)	15 (M) 13 (M)	NH (chamber) N (chamber)	15% FiO_2 20.9% FiO_2	Strength trained	24.6±6.8 23.2±5.2	74.9±11.5 69.4±7.4	8 (2)	3 rounds x 2 blocks x 3 sets x 6RM (85%RM) (Rest 35s bt. exercises, 180 s bt. sets, 5 min bt. blocks)	Bench press Leg extension Front pull down Deadlift Preacher curl Calf raises		Muscle thickness
Törpel (2020)	20 (3F, 17M) 17 (2F, 15M)	NH (facemask) N (facemask)	80-85% SpO_2 20.9% FiO_2	Untrained	24.5±4.5 24.0±3.6	75.5±7.8 76.3±9.2	5 (4)	3 sets x 15 reps. 25-40% RM (Rest 30 s)	2 training plans with 8 machine-based resistance exercises		Lean mass
van Doorslaer de ten Ryen (2021)	10 (M) 9 (M)	NH (chamber) N (chamber)	13.5% FiO_2 21% FiO_2	Untrained	21.2±0.5 20.7±0.8	73.3±3.0 71.1±3.1	4 (3)	6 sets x 10 reps. 80% RM (Rest 120 s)	One leg extension	RM	Muscle thickness
Yan (2016)	8 (M) 9 (M) 8 (M)	NH (room) NH (room) N (room)	12.6% FiO_2 16% FiO_2 21% FiO_2	Rec. trained	22.2±2.6	70.5±10.0	5 (2)	5 sets x 10 reps. 70% RM (Rest 60 s)	Back squat	RM	Lean mass

n: sample size; H: hypoxia; N: normoxia; NH: normobaric hypoxia; FiO_2: fraction of inspired oxygen; SpO_2: arterial oxygen saturation; s/w: sessions per week; reps.: repetitions; Bt: between; Rec.: recreationally; RM: repetition maximum; CSA: cross-sectional area; M: male; F: female.

Consistent with previous meta-analytic approaches[313], we chose to avoid drawing binary conclusions via traditional null hypothesis significance testing given the documented issues with this statistical method[314,315]. Rather, we considered the spectrum of possible estimates from the lower to upper limits of compatibility, placing the greatest inferential emphasis on the point estimate. Threshold values for SMDs were interpreted as: "trivial" (≤0.20); "small" (0.21–0.50); "medium" (0.51–0.80); and "large" (>0.80)[316].

For SMDs with a positive value, the reported result favors the RTH; conversely, results with a negative value favor RTN. The Q test and I^2 index were calculated to estimate potential statistical heterogeneity. A threshold from 30 to 60% represented a moderate level of heterogeneity, $p<0.10$. Potential small study bias was analysed using Egger's test[317] as estimated from a funnel plot (Figure S2). A sensitivity analysis was performed to control for the robustness in the outcomes that included the studies eliminated for undetermined inter-set rest periods[318] . All statistical analyses were performed using the Metafor package[319] in the R statistics program[320]

Section II. Preliminary studies on acute exposure to hypobaric hypoxia.

Studies 2 and 3 compared the acute effects of a hypertrophy-oriented R_T session under terrestrial hypoxia. The following section is divided into the common subheadings for both studies (Experimental approach to the problem, Participants, Hypertrophic resistance training session and Blood extractions) and the different measurements performed on each study:

» Experimental Approach to the Problem

In a counter-balanced order, participants performed two traditional hypertrophic-oriented R_T sessions, one at moderate-altitude (HH; 2320 m asl) and the other under normoxic conditions (N; <700 m asl). One week before the first R_T session, participants were required to complete a preliminary session to determine their 10-repetition maximum (10RM) load in each exercise at a normoxic environment. Then, 72 hours before the start of the study and after 48 hours of rest at N, participants attended the laboratory for anthropometric [height (Seca 202, Seca Ltd., Hamburg, Germany), body mass (Tanita BC 418 segmental, Tokyo, Japan)] and resting blood sample testing (Figure 11).

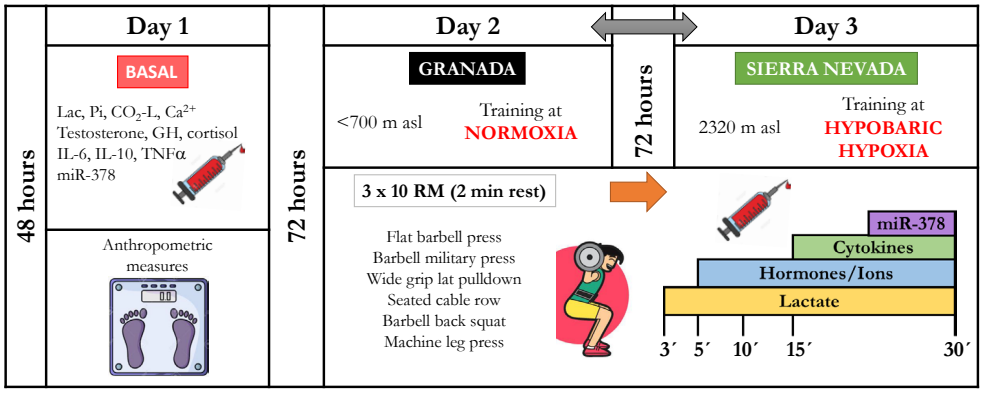


Figure 11 — Study design.

During the experimental period, participants fasted after midnight of the day before each training session. They were provided with a meal replacement supplement (610 calories; 31% protein, 41% carbohydrate and 12% fat) 1.5 hours prior to the start of the corresponding warm-up. Testing sessions

were conducted at the same time of day, at a temperature of ~22º C and ~60% humidity, or ~22º C and ~28% humidity for N and H conditions, respectively. Participants travelled by car to the altitude training center (32 km) to perform each training session. Arrivals to altitude occurred ~30 min before the training session and participants returned to normoxia after completing the session. Arterial oxygen saturation (SpO_2; Wristox 3100; Nonin, Plymouth, MN, USA) was evaluated before the start of the warm-up of each training session to test the H condition. Participants displayed a mean SpO_2 value of 94.2±1.3 and 98.0±1.5% in H and N, respectively ($p<0.001$).

» Participants

Thirteen male volunteers (age: 22.31 ± 2.59 years; height: 178.31 ± 4.96 cm; body mass: 76.92 ± 9.17 kg) participated in the study. All participants had participated in a resistance training regimen for a minimum of 3 times per week for at least 12 months. Subjects had no health or muscular disorders, reported to be free from consumption of any agents associated with increased muscle size during the previous month and had not been exposed to altitudes above 1500 m asl for more than 3-4 consecutive days for at least two months before the study onset. Participants lived at a low altitude to ensure that responses were specific to acute hypoxia exposure. This study was approved by the Local University Research Ethics Committee and conducted in accordance with the Helsinki Declaration. Informed written consent was obtained from all participants prior to beginning the study.

» Hypertrophic Resistance Training session

The R_T sessions comprised six exercises per session targeting major muscle groups of the body (flat barbell press, barbell military press, wide grip lat pulldown, seated cable row, barbell back squat, and machine leg press). A standardized warm-up of 15 min was completed at the beginning of each session consisting of low intensity aerobic exercise and stretching exercises, and a specific warm-up in which they performed two sets of 10 repetitions (the first with 20 kg and the second at 50% 1RM estimated from the preliminary test, 120 s rest) of the back squat, seated cable row and bench press. The routines for each session included 3 sets

of 10 RM per exercise. Subjects rested 2 min between sets. Sets were carried out to the point of momentary concentric muscular failure (inability to perform another concentric repetition while maintaining proper form). Cadence of repetitions was carried out in a controlled fashion, with a concentric action of approximately one second and an eccentric action of approximately two seconds. The load was adjusted for each exercise as needed on successive sets to ensure that subjects achieved failure in the target repetition range (8-10 RM). Barbell exercises were performed with calibrated equipment (Eleiko) and used for comparison between conditions. All routines were directly supervised by the research team to ensure proper performance of the respective routines. Participants were instructed to rest 72 hours between training sessions and to refrain from performing any additional resistance-type or high-intensity anaerobic training throughout the study.

» Blood extractions

Venous blood samples were taken, after each training session. The basal condition was established from a blood analysis collected 2 days prior to the first training session after 48 hours of abstention from structured exercise. All preliminary laboratory assessments and basal blood testing were performed in normoxia.

Immediately following the training session, the antecubital vein of the arm of each participant was canalized via a catheter. The catheter remained permeable by using physiological saline solution. 5 ml of blood was extracted at minutes 3, 5, 10, 15 and 30 post-exercise. An amount of 2 ml of blood before each extraction was discarded to avoid dilution of the sample. In all cases, blood samples were kept at cold conditions and centrifuged in the following 4 hours during 10 min at 3000 rpm. Finally, 500µl aliquots were stored at -70°C until use. Blood extractions were performed at the same altitude condition of the corresponding session by specialized staff (Figure 12).

Figure 12 — Blood extraction, storage and centrifugation.

Study 2. Altitude-induced effects on muscular metabolic stress and hypertrophy-related factors after a resistance training session.

Feriche, B., Schoenfeld, B. J., Bonitch-Góngora, J., de la Fuente, B., Almeida, F., Argüelles, J., Benavente, C., & Padial, P. (2019). Altitude-induced effects on muscular metabolic stress and hypertrophy-related factors after a resistance training session. European Journal of Sport Science, 18, 1-10.

» Measurements

Absolute training load by exercise (kg), repetitions to failure, and ratings of perceived exertion (RPE) were monitoring during and after each training session respectively. RPE was obtained by showing a graphical scale to participants 30 min after completing the training session [321].

Metabolites/ions (lactate, inorganic phosphate, liquid carbon dioxide and calcium) and hormones (testosterone and growth hormone) were analyzed from venous blood samples. Blood lactate concentration (Lac) was assessed at minutes 3, 5, 10, 15 and 30 and analyzed by a photometric procedure (Dr. Lange, LP 20 plus, Berlin, Germany). Ions and hormone values were assessed at minutes 5, 10, 15 and 30 post-exercise and analyzed in a COBAS C-311 System (Roche, Basel, Switzerland). To minimize bicarbonate loss, specimens were kept tightly capped.

» Statistical analysis

Data are presented as mean ± standard deviation (SD). Normal distributions of the data were confirmed using a Shapiro-Wilk test. A two-factor ANOVA was used to assess the effect of time during the recovery (within-participant factor with 4 levels [minutes 5, 10, 15 and 30] and the environmental condition (within-participant factor with 2 levels [HH *vs.* N]) on the ions and hormone variables. A Greenhouse-Geisser correction was employed when the sphericity assumption in ANOVAs was violated (Mauchly's test). Post hoc comparisons, when appropriate, were performed using Bonferroni correction for multiple pairwise comparisons. Generalized Eta-Squared measures of effect size and thresholds

were set as follow: <0.04 (small), >0.04 (medium) and >0.36 (large). Differences with respect to the basal conditions of all blood variables and between condition comparisons for maximal blood lactate concentration, RPE and physical performance were analyzed through paired samples t-tests. To quantify the magnitude of the change, standardized differences (i.e., Cohen's *d* effect sizes) were calculated as the mean change divided by the pooled standard deviations on all dependent variables. Threshold classifications were set as follow: >0.2 (small), >0.6 (moderate), >1.2 (large) and >2 (very large). All analyses were performed using the software package SPSS (version 24.0, IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA). Effects were considered significant at $p \leq 0.05$.

Study 3. Hormonal and Inflammatory Responses to Hypertrophy-Oriented Resistance Training at Acute Moderate Altitude.

Benavente, C., León, J., Feriche, B., Schoenfeld, B. J., Bonitch-Góngora, J., Almeida, F., Perez-Regalado, S. & Padial, P. (2021). Hormonal and Inflammatory Responses to Hypertrophy-Oriented Resistance Training at Acute Moderate Altitude. International Journal of Environmental Research and Public Health, 18(8), 4233.

» Blood Measurements

Venous blood samples were taken for determination of hormones (testosterone, growth hormone and cortisol), cytokines (IL-6, IL-10 and TNFα) and miR-378. Hormonal values were assessed at minutes 5, 10, 15 and 30 post-exercise to determine recovery peak values. Analyses were performed in a COBAS C-311 System (Roche, Basel, Switzerland). Cytokine analyses were assessed at minutes 15 and 30 of the recovery by multiple immunoassay kits (Procarta Multiplex immunoassay kit, Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Quantitative data were obtained using the Luminex-200 system (Luminex Corporation, Austin, TX, USA), and data analysis was performed

on Luminex 100™ IS v2.3 software. Assay sensitivity for each cytokine measured was (pg/mL): IL-10: 0.2466, IL-6: 3.74 and TNFα: 0.835. Finally, cell-free total RNA — primarily miRNA — was obtained at minute 30 of recovery by miRNeasy Serum/ Plasma Kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany). A panel of 3 invariant miRNAs, two snoRNAs (SNORD95, SNORD96A) and one snRNA (RNU6-2) were used to normalize for variability in sample loading and real-time RT-PCR efficiency [322,323]. Cycling was performed under standardized conditions with 2x QuantiTect® SYBR Green PCR Master Mix on a CFX96 Real-Time PCR Detection System (Biorad, California, United States) (Figure 13). To determine the efficiency of RNA extractions and/or the presence of inhibitors in cDNA synthesis or in PCR, the levels of the Spike-in Control were quantified (C. elegans miR-39 miRNA mimic) and added before RNA extraction by real-time PCR. The fold changes of candidate miRNAs expression were calculated by the equation $2^{-\Delta\Delta Ct}$. All analysis were performed at normoxia using the same equipment.

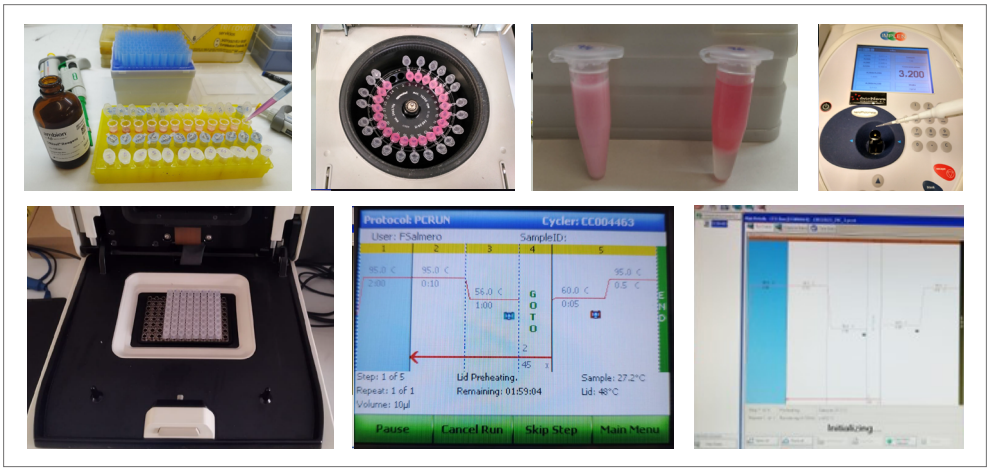


Figure 13 — miRNA quantification through RNA extraction, reverse transcription, and PCR.

» Statistical Analyses

Data are presented as mean ± standard deviation (SD) or mean standard error (SEM). Normal distributions of the data were confirmed using a Shapiro-Wilk test. Cytokines values were dichotomized to 1 or 0 as detected or non-detected data, respectively, with respect to the minimum detectable threshold established by the analysis kit. Differences between conditions (N vs. H) and time (basal vs. min 15 vs. min 30) were interpreted through the McNemar test. A paired sample t-test was used to assess the session effect (basal vs. maximal value) between exercise conditions (N vs. H) in the peak value of the hormones studied. To quantify the magnitude of the change, standardized differences (i.e., Cohen's d effect sizes) were also calculated as the mean change divided by the pooled standard deviations in all dependent variables. Threshold classifications were set as follows: >0.2 [small], >0.6 [moderate], >1.2 [large] and >2 [very large].

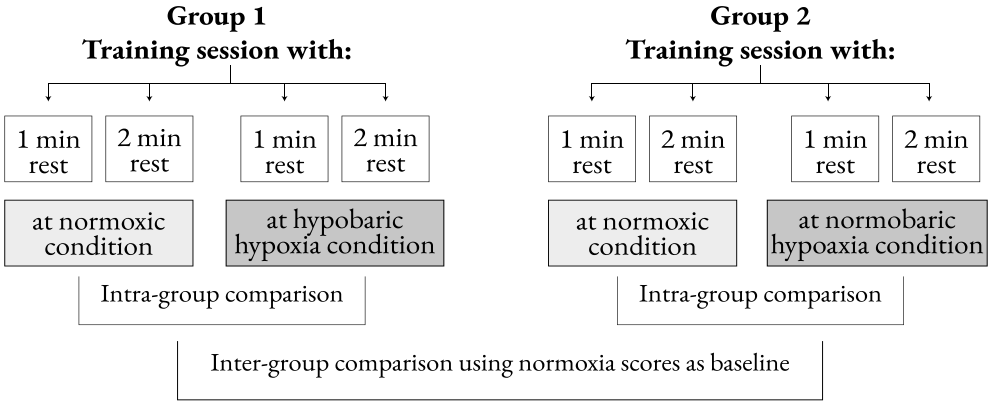
The relationship between the miRNA response and the higher value of the hormones variables was calculated through a Pearson or Spearman correlation coefficient (r) in both conditions. The corresponding Fisher's Z-transformed *r* coefficient was used for N and H comparison by calculating the Fisher's F distribution. Previously, the Spearman's coefficients (rho) were converted to Pearson's coefficients (r) when appropriate. For our sample size (n=13), significance was determined as follow: F>2.69 [p=0.05] and F>4.16 [p=0.01]. The inference analysis was determined by the Cohen's Q effect size calculation (magnitudes were interpreted as follows: <0.1: no effect; 0.1 to 0.3: small effect; 0.3 to 0.5: intermediate effect; >0.5: large effect). A binary logistic regression was carried out to define the relationship between miRNA and cytokines. An indication of the fit of the model was given by Cox-Snell's R^2 and an Omnibus test (Chi-Square). All analyses were performed using the software package SPSS (version 26.0, IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY). Effects were considered significant at $p \leq 0.05$.

Section III. Acute effect and comparison between types of hypoxia.

Studies 4, 5 and 6 compare the acute effects of different types of hypoxia after a hypertrophy-oriented R_T session. The following section is divided into the common subheadings for all studies (Experimental approach to the problem, Participants, Hypertrophic resistance training session and Blood extractions) and the different measurements performed on each study:

» Experimental approach to the problem

A repeated measures model was applied in two independent groups (G1 and G2), one for each type of hypoxia. All participants performed a standard hypertrophic R_T session on four different days, counterbalancing the order in terms of environmental condition (N, HH and NH) and type of inter-set rest (60 and 120 s). Each session was separated by a rest period of 72 h. Thus, participants in G1 performed each of the two inter-set rest types of R_T sessions at normoxia (N) and at terrestrial hypoxia (HH: 2320 m asl; ~ 570 mmHg). Participants in G1 travelled by car to the HH center (32 km), began the training session ~30 min after arrival to altitude and then returned to normoxia after completing the session. Participants in G2 performed the same routines as G1 under equivalent simulated normobaric hypoxia (NH: < 700 m asl; inspired fraction of oxygen [FiO_2] = 16.9%). The study design is illustrated in Figure 14.



* In each group the environmental condition and the inter-set recovery were randomly counterbalanced

Figure 14 — Study design.

One week before the first R_1 session, subjects engaged in a preparatory session to determine their training load (70% of 1RM) for each exercise. This load was the average between two attempts with different loads separated by 15 min. Two days before the beginning of the study, participants attended the laboratory for baseline anthropometric measures (height [Seca 202, Seca Ltd., Hamburg, Germany] and body mass [Tanita BC 418 segmental, Tokyo, Japan]). Preliminary assessments were performed under normoxic conditions and participants were instructed to abstain from physical activity and alcohol intake, and to maintain their customary sleep and diet habits for 48 h before evaluations. To ensure standardized nutritional intake for performance during the R_1 sessions, participants fasted after midnight the evening prior to a training session and were provided with a standardized breakfast (730 kcal) and a protein bar (350 kcal) at 2 h and at 40 min prior to the start of the warm-up, respectively. Exercise was conducted in the morning at the same time of day for all participants under the conditions of ~22º C and ~60% humidity for the N and NH conditions, or ~22º C and ~28% humidity for the HH condition. The hypoxic environmental condition was assessed by the arterial oxygen saturation (SpO_2) measured before the start of the warm-up.

» Participants

Sixteen active, resistance-trained men (G1 [n=9]; age: 23.6±3.2 years; height: 177.2±5.7 cm; body mass: 73.9±5.3 kg and G2 [n=7]; age: 26.0±3.0 years; height: 174.0±5.0 cm; body mass: 73.9±7.8 kg) volunteered to participate in the study. They had no health or muscular disorders, had not ingested substances to increase muscle size for the previous year and were not exposed to more than 3-4 consecutive days of altitudes above 1500 m asl for at least two months before the study. Participants lived at a low altitude to ensure that responses were specific to acute hypoxia exposure. All subjects had been consistently lifting weights for at least 12 months prior to the onset of the study. Before the study, participants were provided with information detailing the purpose and requirements of the research protocol and provided signed informed consent. This study was approved by the Andalusian Government Research Ethics Committee

(Ethical Application Ref: # 1540-n-18) and conducted in accordance with the Helsinki Declaration.

» Hypertrophic resistance training session

Before the training sessions, participants undertook a standard warm-up protocol consisting of 15 min of low intensity aerobic exercise and stretching exercises, and a specific warm-up in which they performed 2 sets of 10 repetitions (the first with 20 kg and the second at 50% 1RM estimated from the preliminary test, 120 s rest) of the back squat, seated cable row and bench press. The R_T session included six exercises that targeted movement patterns involving major muscle groups of the body in the following order: back squat, machine leg press, seated cable row, wide grip lat pulldown, bench press and barbell military press.

Each training session comprised 3 sets of 10 repetitions per exercise with a load of 70% of 1RM and 60 s or 120 s of inter-set and inter-exercise rest. Cadence of repetitions was carried out in a controlled fashion, with a concentric action of approximately 1 s and an eccentric action of approximately 2 s as determined by the supervising researcher. The load was reduced by 5% as needed in those cases where participants reached volitional failure before achieving the target repetition range (8-10 repetitions) with respect to the previous set (i.e., in the 2nd or 3rd set). All routines were directly supervised by the research team to ensure they were properly performed.

Absolute training load by exercise (kg) and repetitions were monitored during each training session. Due to differences in training machine models between locations, only the barbell back squat and bench press were used for comparison. Total volume-load was calculated as the sum of the load lifted × the repetitions x set of each exercise [84]. Before the warm-up of each session SpO_2 was measured in duplicate using a pulse oximeter (Wristox 3100; Nonin, Plymouth, MN, USA). Participants mean rest SpO_2 value equated to 98.4±0.9 and 94.3±0.5% for G1 (N and HH, respectively), and 98.5±0.5 and 90.7±1.0% for G2 (N and NH, respectively).

» Hypobaric-normobaric hypoxia conditions

G1 performed the hypoxic training sessions under terrestrial hypoxic conditions at the High-Performance Center of Sierra Nevada (2320 m asl., Spain). The normobaric hypoxia condition of G2 was carried out by connecting a facial mask to participants 5 min before the start of the warm-up that maintained breathing at a reduced FiO_2 (15.9%) during the hypoxic training sessions. FiO_2 during exercise was controlled using an electronic device (HANDI+, Maxtec, Salt Lake City, Utah, USA). The FiO_2 level was calculated according to the guidelines provided by the hypoxic generator manufacturer to equate an altitude of 2320 m. The low oxygen air was produced by a hypoxic generator with a semi-permeable filtration membrane (nitrogen filter technique; CAT 310, Louisville, Colorado, USA).

» Blood extractions

Venous blood samples were taken, after each training session. The basal condition was established from a blood analysis collected 2 days prior to the first training session after 48 hours of abstention from structured exercise. All preliminary laboratory assessments and basal blood testing were performed in normoxia.

Immediately following the training session, the antecubital vein of the arm of each participant was canalized via a catheter. The catheter remained permeable by using physiological saline solution. 5 ml of blood was extracted at minutes 3, 5, 10 and 30 post-exercise. An amount of 2 ml of blood before each extraction was discarded to avoid dilution of the sample. In all cases, blood samples were kept at cold conditions and centrifuged in the following 4 hours during 10 min at 3000 rpm. Finally, 500µl aliquots were stored at -70°C until use. Blood extractions were performed at the same altitude condition of the corresponding session by specialized staff.

Study 4. Inter-set rest configuration effect on acute physiological and performance-related responses to a resistance training session in terrestrial vs. simulated hypoxia.

Benavente, C., Feriche, B., Olcina, G., Schoenfeld, B.J., Camacho-Cardeñosa, A., Almeida, F., Martínez-Guardado, I., Timón, R., Padial, P. (2022). Inter-set rest configuration effect on acute physiological and performance-related responses to a resistance training session in terrestrial vs. simulated hypoxia. Peer J, 10, e13469.

» Training session monitoring

- *Metabolic and cardiovascular responses.*

Blood lactate concentration (Lac) was assessed before and immediately following the training session, at minutes 3, 5, 10 and 30 using a Lactate Pro 2 device (Arkray, Japan). Basic cardiovascular response was quantified from a heart rate (HR) cardiotachometer (Polar s610i; Polar Electro Oy, Kempele, Finlandia) during all training sessions and over the course of the immediate 30 min post-exercise period. The mean value of HR recorded was classified as working HR (work-HR), rest time between sets HR (rest-HR) and HR along the post-exercise recovery period (HR_{30})

- *Perceptual responses.*

Sessional rating of perceived exertion was obtained via a Category Ratio-10 scale viewed by participants 30 min after completing the training session (RPE-30) [324].

- *Muscle oxygenation.*

Muscle oxygen saturation (SmO_2) was measured by near-infrared spectroscopy (NIRS; Moxy, Fortiori Design, Minneapolis, Minnesota, USA) during the first exercise (back squat) of each training session. The Moxy device measures the total hemoglobin (Hb) present beneath the device, as well as calculates the percentage of Hb containing O_2 (SmO_2)[325]. SmO_2 reflects the dynamic balance between O_2 supply and consumption calculated throughout the change in total tissue oxy (+myo) hemoglobin (O_2Hb) and deoxyhemo- (+myo-) globin (HHb)[326].

The sampling rate of the sensor was 2 Hz. SmO_2 values were expressed in % and calculated as follows by the device:

$$SmO_2(\%) = O_2Hb / [O_2Hb + HHb] \times 100$$

During all testing, the system was connected to a personal computer via a software program (Seego: Realtrack Systems, Spain) that provided a graphic display of the data. The sensor was placed on the vastus lateralis of the participant's dominant leg, halfway between the greater trochanter and lateral epicondyle of the femur, before the warm-up. This position was marked with a semi-permanent pen on the skin to reproduce the exact location in subsequent tests. To avoid issues with movement during exercise, the device was fixed to the leg with tape and wrapped with a dark elastic bandage. Maximal and minimum values were recorded for each set of the exercise (Figure 15). The difference between maximal and minimum values was used to calculate the SmO_2 of the first (SmO_2S_1), second (SmO_2S_2) and third (SmO_2S_3) set. The mean of the three sets was calculated to express the total mean SmO_2 of the exercise (SmO_2T).

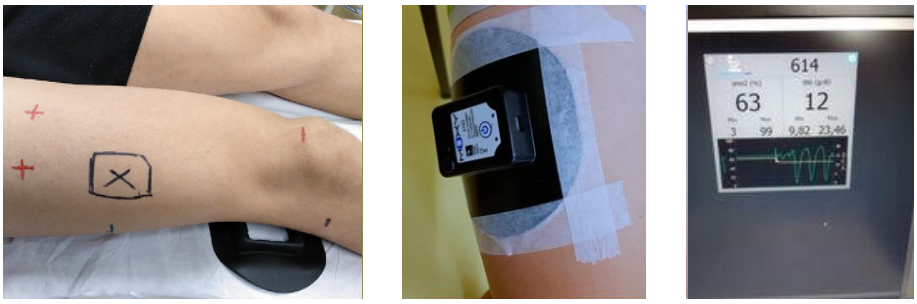


Figure 15 — Muscle oxygenation device placement and registration.

» Statistical analyses

Data are presented as mean ± standard deviation (SD). Normal distributions of the data were confirmed using a Shapiro-Wilk test. A linear mixed-effects model with inter-set recovery (60 s vs. 120 s), environmental condition (HH and NH), and their interaction was applied for analysis. Varied intercepts were permitted by

treating the subject as a random effect. This model was built for the physiological variables. To ascertain the eventual effect of training load on the performance of 2 comparable exercises among conditions (back squat for the lower-limbs and bench press for the upper-limbs), normoxia baseline scores were included as a covariate of no interest [327]. Also, the adjusted between-group difference was calculated as the estimated marginal mean of the difference between HH and NH groups (HH group – NH group) after adjusting for N baseline differences. To quantify the magnitude of the change, we calculated 90% confidence intervals (CIs) of the adjusted effect.

The standardized mean differences (i.e., Cohen's d effect sizes) were calculated as the mean change (H-N or 120-60 s) divided by the pooled standard deviations of the change in all dependent variables or as the adjusted between-group difference divided by the pooled normoxia SD when comparing hypoxia types [328]. Threshold classifications were set as follows: >0.2 [small], >0.6 [moderate], >1.2 [large] and >2 [very large][329].

Consistent with other research in applied sports science[330], we used an estimation-based approach to drawing inferences from our data. Accordingly, we interpreted each effect and its precision continuously331 rather than relying on null hypothesis significance testing[314]. This follows current statistical recommendations to eschew dichotomous interpretations of results in favor of models that provide estimates of practical meaningfulness[332]. All analyses were performed using the software package SPSS (version 26.0, IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY).

Study 5. Effects of resistance training in hypobaric vs. normobaric hypoxia on circulating ions and hormones.

Timón, R., Olcina, G., Padial, P., Camacho-Cardeñosa, A., Bonitch-Góngora, J., Martínez-Guardado, I., Camacho-Cardeñosa, M., Benavente, C., De la Fuente, B., Feriche, B. (2022) Effects of Resistance Training in Hypobaric vs. Normobaric Hypoxia on Circulating Ions and Hormones. International Journal of Environmental Research and Public Health, 19(6), 3436.

» Measurements

Venous blood samples were taken for determination of hormones (growth hormone and cortisol), and ions (Ca^{2+}, HCO_3^-, Pi). Ions and hormone values were assessed at minutes 5, 10 and 30 post-exercise. Ions were analyzed in a COBAS C-311 System (Roche, Basel, Switzerland), and hormones were determined in a COBAS E-411 System (Roche, Basel, Switzerland), which was calibrated daily. The COBAS system includes calibration systems for each batch of reagents, and these are validated by comparison with predefined limits for each analyte. The kits or panels that were tested in COBAS had previously been validated by the commercial company and offered the coefficient of variation (CV) of the protocol (CV < 3%).

Blood lactate concentration from the antecubital vein was analyzed by using Lactate Pro 2 (Arkray, Japan) at 3-min post-exercise. The maximum value was registered in mmol/L.

Rating of perceived exertion (RPE) was obtained by showing a graphical scale to participants 30 min after completing the training session[333], with a Category Ratio-10 scale.

» Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 27.0, Chicago, IL, USA). The Shapiro–Wilks test was applied to verify a normal distribution of data, and Levene's test was used to assess the homogeneity of variance. A multifactorial ANOVA of repeated measures (within-group factor with four levels: baseline, 5, 10, and 30 min) was independently

performed, analyzing the main effects and interaction effects of inter-set rest factor (1-min vs. 2-min) and hypoxic environmental condition factor (HH vs. NH) on the variables. Post-hoc Bonferroni tests were performed when appropriate for multiple comparisons. The effect size (ES) was calculated for all dependent variables, taking into account the dispersion of the group means during the different time points. Additionally, a one-way ANOVA was used to compare participant characteristics and control variables. The significance level was set at $p \leq 0.05$, with a confidence level of 95%. Means and standard deviations (SD) were used as descriptive statistics.

Study 6. Influence of the terrestrial vs. simulated hypoxia on biomolecular anabolic signalling response to resistance exercise: A pilot study.

Feriche, B., Benavente, C., Olcina, G., Padial, P., Argüelles, J., Camacho-Cardenosa, M., Timón, R., León, J. (2023). Influence of the terrestrial vs. simulated hypoxia on biomolecular anabolic signalling response to resistance exercise: A pilot study. European Journal of Applied Physiology. [Submitted]. Impact factor: 3.346, Q2 in Sport Sciences.

» Blood measurements

Venous blood samples were taken for determination of cytokines (irisin and myostatin), hormones (growth hormone and cortisol), miRNAs (miR-378, miR-260 and miR-29c) and blood lactate. Cytokine values were assessed 5- and 30-min post-exercise. ELISA kits were used for myokine analysis [Myostatin ELISA (R&D Systems) and Irisin (Phoenix Pharmaceutical) kits] (Figure 16).

Micro-RNAs were assessed 30 min post-exersice. total cell-free RNA – mainly miRNA – was obtained at minute 30 of recovery by miRNeasy Serum/Plasma Kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Reverse transcription was performed with an miScript II RT (Qiagen) kit. Quantitative PCR was carried out under standardized conditions with 2× QuantiTect® SYBR Green PCR Master Mix (Qiagen) in a real-time PCR detection system

CFX96 (BioRad, California, USA). For relative miRNA quantification, a synthetic non-human miRNA, cel-miR-39, was used as a spike-in control added during RNA extraction. The changes in the fold of the expression of the candidate miRNAs were calculated using the equation $2-\Delta\Delta^{Ct}$. Hormonal and blood lactate values were assessed 30 post-exercise. Hormones were analyzed in a COBAS C-311 System (Roche, Basel, Switzerland) and blood lactate was analysed using a Lactate Pro 2 (Arkray, Japan).

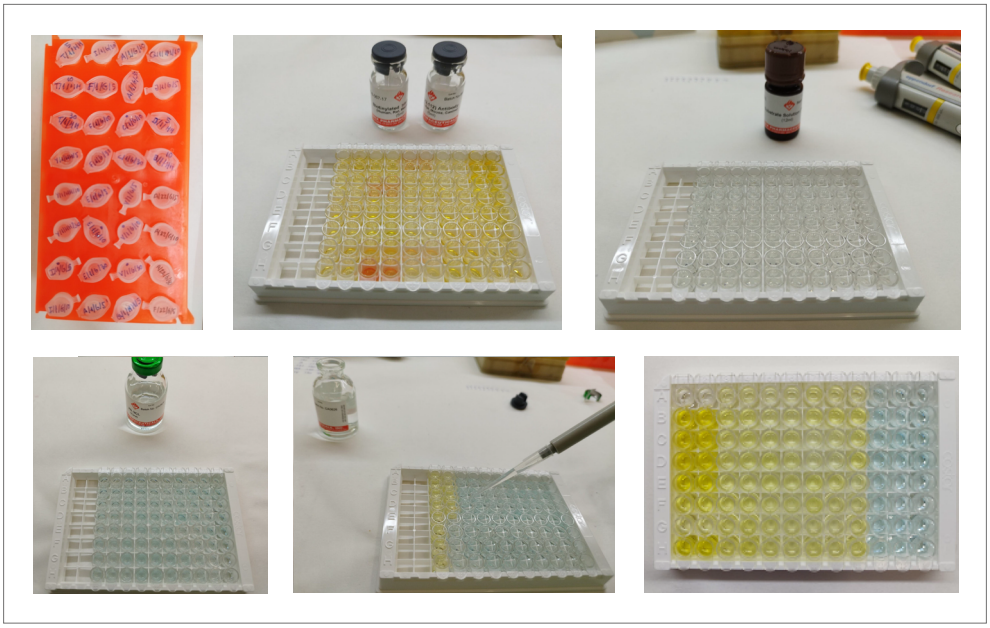


Figure 16 — Myostatin and irisin analysis through ELISA kit.

» Statistical analysis

Data are presented as mean or mean difference (MD) ± standard error of the mean (SEM). For mean difference calculation, corresponding baseline scores were used (post – pre or HH – NH or H – N). Normal distributions of the data were confirmed using a Shapiro–Wilk test. If needed, we applied a data transformation process on non-normal variables (the square of the natural logarithm in myostatin, miR-378 and miR-29c; natural logarithm in GH). A linear mixed-effects model was applied with the environmental conditions as a fixed effect (N vs. HH vs.

NH); the moment of sample collection (rest vs. 5 min vs. 30 min) as a random effect when corresponded; and their interaction. Tukey's test was applied as post hoc analysis when significant differences were noted.

The magnitude of the change was calculated by using 90% compatibility intervals (CIs) of the adjusted effect. The adjusted standardized mean differences (i.e., effect size) were also calculated as the adjusted mean change (HH – NH or H – N) divided by the pooled standard deviations in all dependent variables. Values were adjusted by the corresponding pre-exercise value. Threshold classifications were set as follows: > 0.2 [small], > 0.6 [moderate], > 1.2 [large] and > 2 [very large][329].

Similar to previous work, we used an estimation-based approach to draw inferences from our data. Specifically, rather than relying on null hypothesis significance testing and drawing binary conclusions as to the presence of an effect or no effect[314], we interpreted each effect and its precision continuously[261].

All analyses were performed using the software package SPSS (version 26.0, IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA).

Section IV. Chronic effect and comparison between types of hypoxia.

Study 7. Strength and muscle mass development after a resistance-training period at terrestrial and simulated intermittent hypoxia.

Strength and muscle mass development after a resistance-training period at terrestrial and simulated intermittent hypoxia. [Under writing process].

» Experimental approach to the problem

A longitudinal design with inter- and intra-group measurements was employed to analyze the influence of the type of moderate hypoxia (terrestrial vs. simulated) on strength, muscle mass and related serum biomarkers response after an 8-week R_T program with respect to the same training in normoxia condition. Participants were randomly assigned to normoxia (N; FiO_2 = 20.9%; ~760 mmHg), hypobaric hypoxia (HH; 2,320 m asl; ~570 mmHg) or normobaric hypoxia (NH; FiO_2 = 15.9%; ~760 mmHg). All participants lived permanently under normoxia conditions. The week before starting the training, participants visited the laboratory to determine the training load. Seventy-two hours before and after the study, and after 48 h of rest, participants were measured for anthropometric (height [Seca 202, Seca Ltd., Hamburg, Germany], body weight [Tanita TBC-300, Tokyo, Japan], muscle thickness and resting blood sample testing determination. In addition, blood samples were also taken throughout the initial 30 min after the first (S1) and the last (S22) R_T session of the program.

During the study, all individuals agreed to adhere to the prescribed R_T during the 8 weeks of the program, with no intense or systemic exercise performed other than prescribed. Participants were instructed to maintain their habits and regular dietary consumption during the entire measurement and training phases. They were provided with a protein shake supplement (111 kcal per serving) after each session to ensure standardized nutritional intake.

All the sessions were conducted at the same time of day. The hypoxic environmental conditions were assessed by arterial oxygen saturation during the 5 min before the start of

the warning-up (SpO_2; Wristox 3100; Nonin, Plymouth, MN, USA). Participants mean rest SpO_2 value equated to (97.2±1.3; 93.9±1.6 and 94.8±2.2%) for S1 and (98.1±1.5; 94.6±1.3 and 94.7±1.9%) for S22 (N, HH and NH respectively).

» Subjects

Thirty-three strength-trained males participated in the study. The subjects were assigned to one of the 3 training groups: the NR_T group lived and trained in N (n=10, age: 22.7 ± 3.4 years; height: 175.3 ± 4.1 cm; body mass: 72.0 ± 7.2 kg); the HHR_T group lived in N and trained in HH (n=10, age: 22.8 ± 4.2 years; height: 177.5 ± 7.4 cm; body mass: 74.0 ± 13.9 kg); and the NHR_T group lived in N and trained in NH (n=13, age: 21.9 ± 2.2 years; height: 176.5 ± 7.4 cm; body mass: 75.0 ± 8.9 kg). All participants had experience in strength training and had participated in a R_T regimen for a minimum of 3 times per week for at least the previous 2 years. Subjects were healthy, had no muscular disorders and reported to be free from consuming any agents associated with increased muscle size during the previous month. All the participants were sea-level residents and had not been exposed to an altitude or hypoxia environment for more than 1500 m asl within the 2 months before the study. This study was approved by the Local Research Ethics Committee and conducted following the Helsinki Declaration. Informed written consent was obtained from all participants before beginning the study.

» Hypoxic exposure

The HHR_T group performed the training sessions under terrestrial hypobaric hypoxic conditions at the High-Performance Center of Sierra Nevada (2320 m asl., Spain). On every training day, participants travelled by car to the altitude center (32 km). Arrivals occurred approximately half an hour before the training session started and they immediately returned to normoxia after completing it. The NHR_T performed the training under simulated hypoxia in a normobaric tent (CAT 430, Colorado Altitude Training, USA). The hypoxic generator system pumped the air through a semi-permeable filtration membrane (nitrogen filter technique; CAT 310, Louisville, Colorado, USA) depleting the oxygen content until reaching a FiO_2 = 15.9 %, according to the manufacturer

guidelines to equate an altitude of 2320 m. Ambient O_2 was continuously monitored by a digital controller (Handi+, Maxtec, Salt Lake City, Utah, USA) to maintain the hypoxic conditions in the tent. Consistent with conventional routine[68] NHR_T participants entered the tent and seated for 5 min to adapt to the training environment before the warming started (Figure 17).

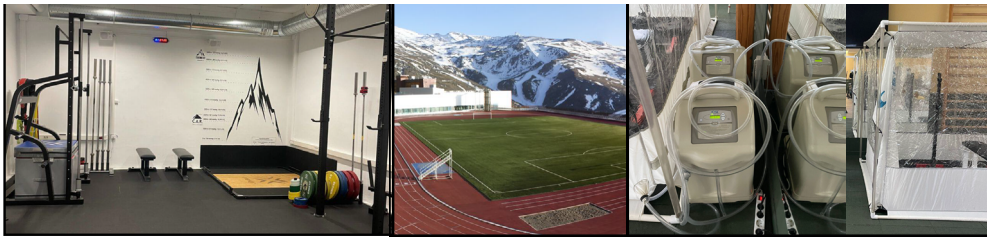


Figure 17 — Training places for NRT, HHRT and NHRT groups.

» Resistance training program

Subjects participated in an 8-week R_T program with 3 sessions/week performed on nonconsecutive days plus an extra rest day at the end of the week. The manipulation of exercise variables (exercise, repetition/set and training load) was designed to elicit a similar mechanical tension throughout the study and greater metabolic stress. Each training session comprised a full-body routine of 3 sets of 6-12 repetitions per exercise (2 additional repetitions to momentary failure in the first set)[334] with a load ranging from 65-80% 1RM and 90 s of rest between sets and exercises[335]. According to the full-body methodology, the 6 exercises of each session developed the same basic movement patterns. The training load and repetition/set modulates throughout the week (i.e., 10 rep/set at 70% 1RM was used in each first session week, 6 rep/set at 80%1RM in the second and 12 rep/set at 65% 1RM in the third). The training load was individually adjusted when participants exceeded the target repetition range while using a proper technique by increasing the training load in accordance (~5%)[21]. A detailed description of the training program (i.e., exercises, sets, repetitions, load, rest periods, etc.) is shown in Table 7. All routines were directly supervised by the research team.

» One Repetition maximum

One RM was determined for each exercise according to the National Strength and Conditioning Association[336]. In brief, prior to the testing, participants performed a warming-up consisting of light cardiovascular exercise lasting 5-10 minutes followed by a set of 5 repetitions at ~50% of the perceived 1RM and after, 1-2 sets more of 2-3 repetitions at a load corresponding to ~60-80% of the perceived 1RM of the exercise. Three sets of 3-6 repetitions at increasing loads were completed before performing 1 set of 2-3 repetitions to failure. The 2-3RM load was used for 1RM estimation from the validated Brzycki's equation[337,338]. Between each successive attempt participant rested for 5 minutes. All 1RM determinations were made within 3 attempts. After the R_T program, subjects repeated the same procedure for the back squat (1RMSQ) and the bench press (1RMBP) for upper- and lower-body comparison.

» Muscle thickness

Individual muscle thickness of the quadriceps' dominant leg was measured using ultrasound equipment (GE-LOGICQ-E portable model; GE Healthcare, Little Chalfont, UK) before and after the training period. According to Miyatani et al.,[339] the maximum thickness of the rectus femoris (RF) and vastus lateralis (VL) was obtained at 50% of the distance from the superior and middle tip of the patella to the anterior superior iliac spine. The lateral location of the VL measurement was taken at 10% of the thigh circumference in the lateral direction. With the subject laid supine, the ultrasound probe (12 L linear probe at 10 MHz frequency, gain 80 dB, depth 8 cm) was orientated perpendicular to the muscle fascicles and the skin, with sufficient ultrasound gel to reduce muscle compression. The specialist adjusted the depth of the image until the femur and muscle boundaries were visible on the screen. Three images were alternatively taken of each muscle and saved in an identified format for subsequent analysis. The average of the measures from the 3 images was used for analysis. The images were retrieved from the ultrasound unit in Digital Imaging and Communications in Medicine (DICOM) format. The thickness of the VL and RF was defined as the distance

Table 7 — Resistance training program during the 8 weeks.

Week 1			Weeks 2-3	
Session 1	**Session 2**	**Session 3**	**Sessions 4 and 7**	**Sessions 5 and 8**
Exercises	**Exercises**	**Exercises**	**Exercises**	**Exercises**
Back Squat Deadlift Pulldown prone grip Barbell Row Bench Press Shoulder Press	Bulgarian dumbbell squat Pulldown narrow grip Dumbbell Bench Press Romanian Deadlift One Arm Dumbbell Row Dumbbell Lateral Raise	Front Squat Pulldown supine grip Bench Press Deadlift Barbell Row Dumbbell Shoulder Press	Back Squat Deadlift Pulldown prone grip Barbell Row Bench Press Shoulder Press	Bulgarian dumbbell squat Pulldown narrow grip Dumbbell Bench Press Romanian Deadlift One Arm Dumbbell Row Dumbbell Lateral Raise
Method	**Method**	**Method**	**Method**	**Method**
3 set x 10 reps. (70%RM), 90 s rest.	3 circuits x 12 reps. (65%RM), 90 s rest.	3 circuits x 10 reps. (70%RM), 90 s rest.	3 set x 10 reps. (70%RM), 90 s rest.	3 set x 12 reps. (65%RM), 90 s rest.

Weeks 2-3	Weeks 4-8		
Sessions 6 and 9	**Sessions 10, 13, 16, 19 and 22**	**Sessions 11, 14, 17 and 20**	**Sessions 12, 15, 18 and 21**
Exercises	**Exercises**	**Exercises**	**Exercises**
Front Squat Pulldown supine grip Bench Press Deadlift Barbell Row Dumbbell Shoulder Press	Back Squat Deadlift Pulldown prone grip Barbell Row Bench Press Shoulder Press	Front Squat Pull down supine grip Bench Press Deadlift Barbell Row Dumbbell Shoulder Press	Bulgarian dumbbell squat Romanian Deadlift Pulldown narrow grip One Arm Dumbbell Row Dumbbell Bench Press Dumbbell Lateral Raise
Method	**Method**	**Method**	**Method**
3 set x 10 reps. (70%RM), 90 s rest.	3 set x 10 reps. (70%RM), 90 s rest.	3 set x 6 reps. (80%RM), 90 s rest	3 set x 12 reps. (65%RM), 90 s rest.

RM: repetition maximum; reps: repetitions.

from the subcutaneous adipose tissue-muscle interface to either the aponeurosis or the muscle-bone interface. The same expert carried out all ultrasound measurements (CV < 1,8%).

» Blood measurements

Participants attended to the laboratory 72 h before the first training session and 48 h after the last training session under fasting conditions for resting blood sample collection in normoxia conditions. In addition, immediately after the first and the last training session at the corresponding environmental condition (S1 and S22), the antecubital vein of the arm of each participant was canalized via a catheter for blood collection. The catheter remained permeable by using a physiological saline solution. Five millilitres of blood were extracted at minutes 5, 10, and 30 post-exercise. We discarded 2 mL of blood before each extraction to avoid dilution of the sample. In all cases, blood samples were kept at cold conditions and centrifuged in the following 4 h for 10 min at 3000 rpm. Finally, 500µl aliquots were stored at −70 °C until use. Venous blood samples were taken for determination of growth hormone (GH), insulin-like growth factor 1 (IGF-1), mammalian target of rapamycin (mTOR), tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) and miR-378/206/29c. All blood extractions were performed by specialized staff.

The analyses followed the manufacturer's instructions using the same equipment. Blood lactate was determined at minutes 3, 5, 10 and 30 of recovery using a Lactate Pro 2 (Arkray, Japan). Growth hormone, IGF-1 and cortisol were assessed at minutes 5, 10, and 30 of the recovery in a COBAS C-311 System (Roche, Basel, Switzerland). IL-6 and TNF-α were assessed at minutes 5 and 30 of the recovery using the Milliplex Human High Sensitivity T Cell Panel (HSTCMAG-28SK) from Sigma-Aldrich (Darmstadt, Germany). mTOR, phosphor-mTOR (Ser2448) and cell-free total RNA—primarily miRNA—were assessed 30 min post-exercise. mTOR and phosphor-mTOR were measured in a Luminex machine using the Procartaplex™ Multiplex Immunoassay from Thermo Fisher Scientific (Vienna, Austria). Briefly, 25 µL of serum were tested in single replicates in 96-well plates. Each plate contained

duplicated serial dilutions (1:4) of a standard sample of known concentration for each analyte provided by the vendor, as well as two blank controls and a reference sample control in duplicate for quality control purposes. Standard curves were used to extrapolate the concentration of the samples, after fitting into a 5-parameter curve algorithm with the LEGENDplex™ Data Analysis Software. The percentage of the active mTOR with respect to the total mTOR was calculated (% active mTOR = phospo mTOR/total mTOR x 100) and used in the analysis. Finally, cell-free total RNA—primarily miRNA—was obtained by miRNeasy Se-rum/Plasma Kit (Qiagen, Hilden, Germany). Reverse transcription was performed with a miRCURY LNA RT kit (Qiagen). Quantitative PCR was carried out under standardized conditions with 2×miRCURY LAN® Master Mix SYBR Green (Qiagen) in a real-time PCR detection system CFX96 (BioRad, California, USA). For relative miRNA quantification, a synthetic non-human miRNA, cel-miR-39, was used as a spike-in control added during RNA extraction. The changes in the fold of the expression of the candidate miRNAs were calculated using the equation $2-\Delta\Delta^{Ct}$.

» Statistical analysis

Data are presented as mean ± standard deviation (SD) or mean standard error (SEM). Before analyzing the study's variables, data normality assumption was tested using the Shapiro–Wilk test ($p > 0.05$). Those variables that showed rates of rejection of normality were subjected to a transformation process.

A repeated measures ANOVA was used to assess the effect of time (i.e., Δ week 8 [week 8 - pretraining] vs. Δ week 6 [week 6 - pretraining] or ΔS1 [session 1- pretraining] vs. ΔS22 [session 22 - pretraining]), the environmental condition (N vs. HH vs. NH), and the interaction between the time x environmental condition on 1RM, muscle thickness, GH, IGF-1, blood lactate, mTOR, IL-6, TNF-α and miR-206. Effect sizes through the partial eta-squared (η^2_p) value and thresholds (0.02 [small], 0.13 [medium] and 0.26 [large]) were calculated along with ANOVA effects (Bakeman, 2005). Non-normally distributed variables (miR-378 and miR-29c) were

compared similarly but using the Kruskal-Wallis (within-subjects) or Wilcoxon test (between-subjects) [340]. Effect sizes through the epsilon squared (ε^2) value and thresholds (0.04 [weak], 0.16 [moderate], 0.36 [relatively strong], 0.64 [strong] and 1.00 [very strong]) were calculated along with Kruskal Wallis effects. A Bonferroni post hoc test was used to analyze pairwise comparisons.

Cohen's effect size (ES) was calculated according to the formula d=(M2– M1/SDpooled), where M1 and M2 are the means of the two groups and SDpooled is the pooled standard deviation (n is sample size and s^2 is variance):

$$spooled = \sqrt{\frac{(n_2 - 1)s_2^2 + (n_1 - 1)s_1^2}{n_1 + n_2 - 2}}$$

ES and the mean difference with 90% confidence intervals (CI) were determined for all pairwise comparisons and interpreted as: < 0.20, trivial; 0.20 to 0.59, small; 0.60 to 1.19, moderate; 1.20 to 1.99, large; and > 2.0, very large[329]. All analyses were performed using the software package SPSS (version 26.0, IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA) and Jamovi[341]. Effects were considered significant at $p \leq 0.10$.

4

Results and Discussion

Section I. Systematic review and meta-analysis.

Study 1. Efficacy of resistance training in hypoxia on muscle hypertrophy and strength development: a systematic review with meta-analysis.

RESULTS

» Study selection

The systematic search returned 743 studies. After removal of duplicates and screening by title and abstract, 49 full-text documents were selected for possible inclusion in the meta-analysis. Two of these studies [342,343] were found to be redundant since they came from the same experiment-i.e., the Manimmanakorn et al.[344] and Martínez-Guardado et al.[345] studies, respectively, provided the same data. Another study was subsequently included because it reported additional muscle thickness data[343]. Ultimately, 17 studies (9 from the previous meta-analysis and 8 new studies) met the pre-determined inclusion criteria. A PRISMA flowchart of the search process is shown in Figure 18.

» Study characteristics

The total sample comprised 348 participants (n = 164 for RTN and n = 184 for RTH). These 17 studies assessed changes in muscle hypertrophy (n=83 for CSA[299,344,346]; n=184 for lean mass[347–349] and n=60 for muscle thickness[292,343,350]) and/or strength development (n=232 for 1RM[70,300,345,351]).

Three studies reported different rest intervals between sets and exercises[343,344,352] and 1 did not specify the inter-set rest interval[351]; thus, all were excluded from the subanalysis of the inter-set rest factor. One study displayed 2 different levels of hypoxia (severe and moderate)[70] and both hypoxia comparisons to normoxia were included in the subanalysis of the severity of the hypoxia.

All included studies employed a live low-train high (live in normoxia-train in hypoxia) strategy, were published between 2003 and 2022, and had sample sizes ranging from 12 to 37 participants. The mean age of participants ranged from 20.2±3.3[344] to 29.0±5.0[349] years old, with body weights ranging from 60.2±16[292] to 98.7±12.8 kg[351]. Only 2 studies totally[344] or partially[348] included women in their samples. The hypoxic condition was simulated in all studies.

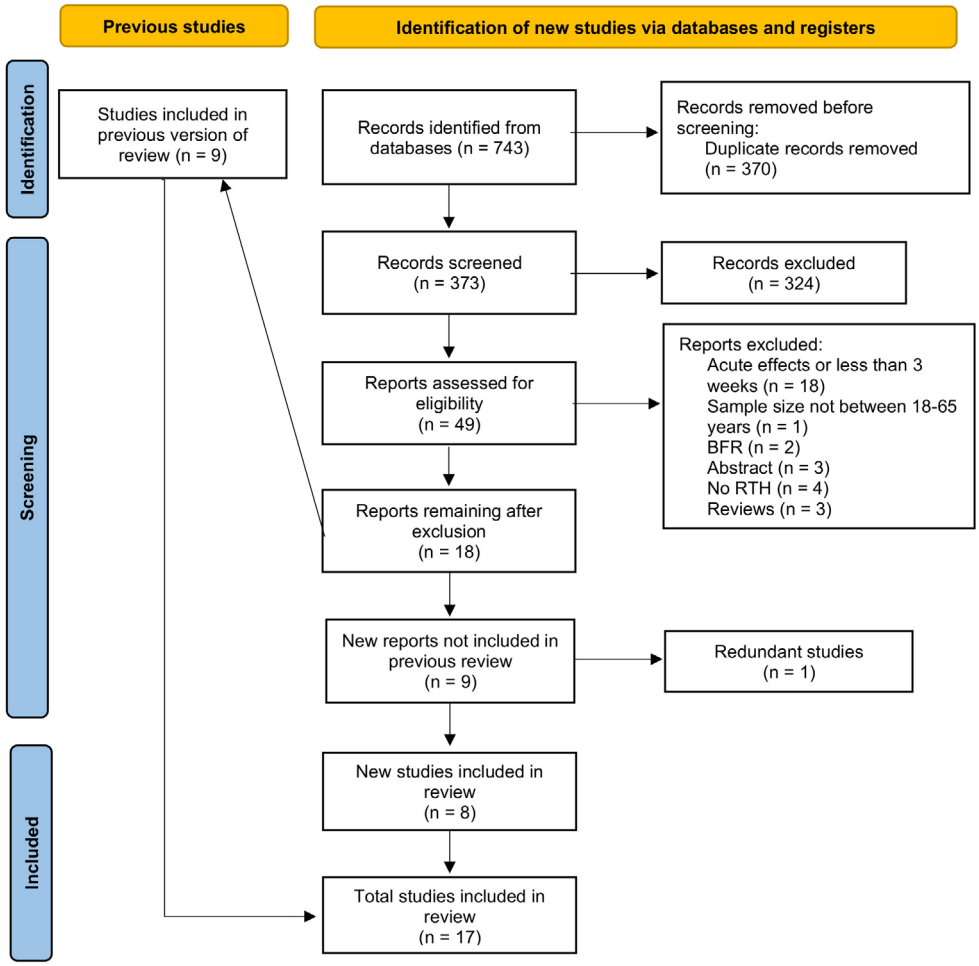


Figure 18 — Flow diagram of the search and selection of studies.

The training status of the sample varied widely across studies with inconsistent terminologies ranging from untrained, recreational, recreationally trained, recreationally strength trained, trained and strength trained to well-trained in a sport discipline and professional context. Given the ambiguity in terminology, we were unable to subanalyze the potential effects of training status on outcomes. Exercise program periods ranged from 3[351] to 8 weeks[292,343,352,353] with a mean training frequency of 2-4 sessions per week. Seven studies used lower limb exercises[70,300,344,346,350,354,355], 2 studies

used single-joint arm flexion and extension exercises[292,299], 3 studies employed a combination of multi-joint upper and lower body exercises[347,349,353], and 5 studies used a full-body routine[343,345,348,351,352].

Nine studies were conducted at moderate hypoxia[70,299,300,343,349,351–353,355] and 9 at severe hypoxia[70,292,344–348,350,354]. Four studies employed the use of low-loads (20-50% of 1 RM)[344,346,348,354], and 3 implemented heavy-load training programs (>80% of 1 RM)[343,351,352]; the remainder of the studies employed moderate-load programs (60-80% 1RM)[70,300,349]. Seven studies used short inter-set rest intervals (≤60s)[70,292,299,344,346,348,354], 3 used moderate inter-set rest intervals (>60-<120s)[345,349,353] and 4 used long inter-set rest intervals (≥120 s)[300,347,350,355].

» Meta-analyses

- *Effect of RTH on muscle hypertrophy*

In the basic analysis, trivial differences in CSA favored RTH over RTN conditions (SMD=0.17 [-0.07; 0.42]; Figure 19). Subanalysis indicated a small effect on CSA benefiting RTH with the use of moderate hypoxia (SMD=0.32 [-0.08, 0.73]; Figure 20A) and moderate loads (SMD=0.32 [-0.08, 0.73]; Figure 20B) and a small effect for short inter-set rest intervals (SMD=0.21 [-0.05; 0.47]; Figure 20C).

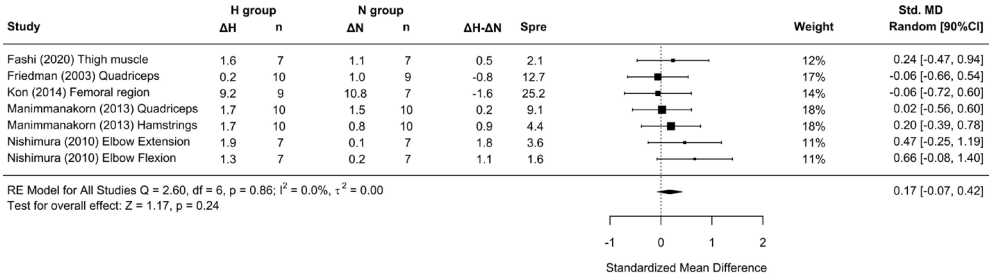


Figure 19 — Forest plot of the standardized mean differences of the total effect of the resistance training program between-conditions (hypoxic [H] group vs. normoxic [N] group) on CSA. Δ: mean differences between post-pre in H and N or between H-N; n: sample size; Spre: mean baseline standard deviation; Std. MD: standard mean difference; RE: random effect's model; CI; confidence interval; Q: test statistic for the test of heterogeneity; df: degrees of freedom; p; p value; I2: I2 test; τ2: tau^2 test; Z: z value.

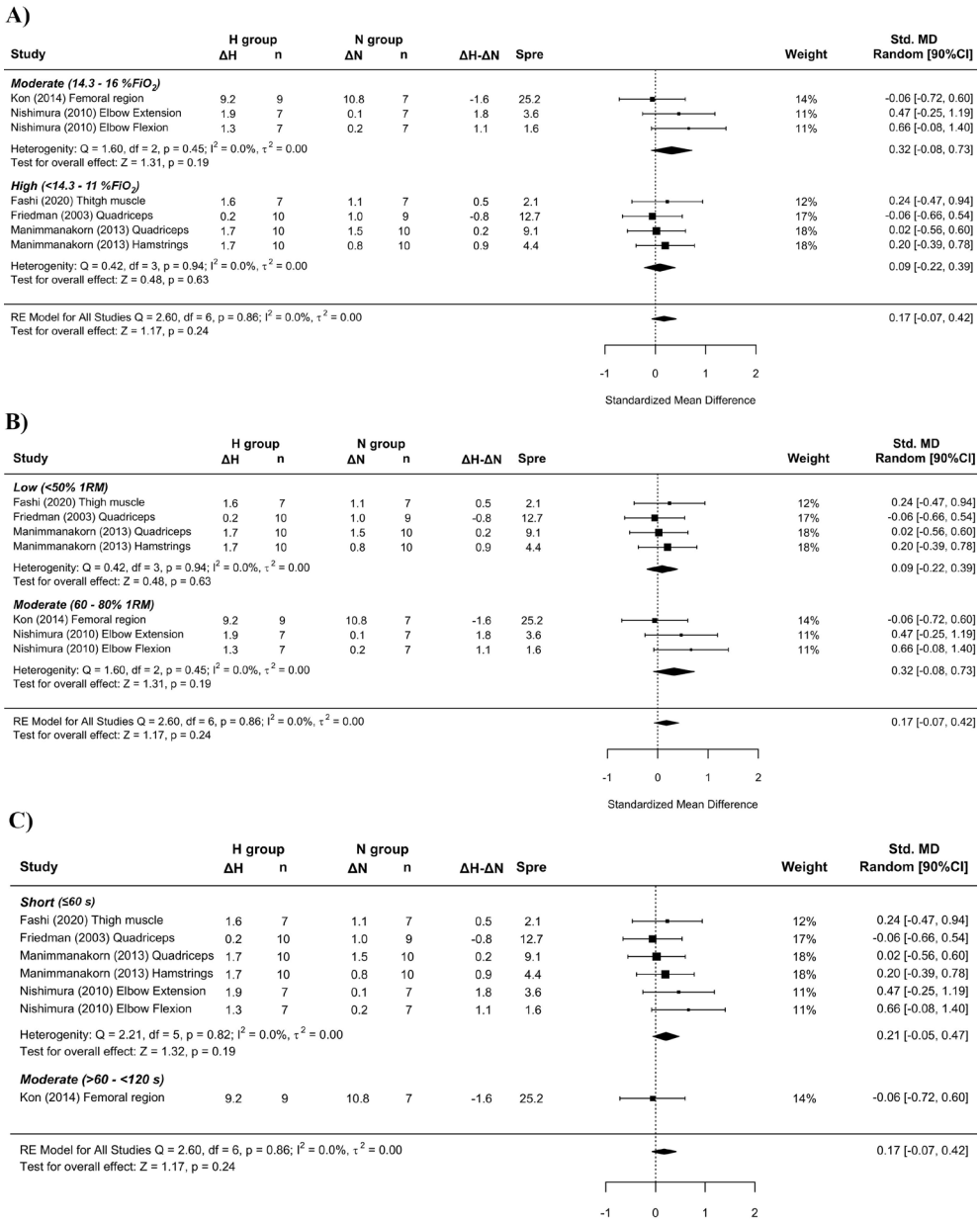


Figure 20 — Forest plot of the standardized mean differences of the resistance training program between-conditions (hypoxic [H] group vs. normoxic [N] group) on CSA, subanalysed by: A) severity of the hypoxia; B) training load; and C) interset rest interval. Δ: mean differences between post-pre in H and N or between H-N; n: sample size; Spre: mean baseline standard deviation; Std.

MD: standard mean difference; RE: random effect's model; CI; confidence interval; FiO_2: fraction of inspired oxygen; 1RM; 1 repetition maximum; Q: test statistic for the test of heterogeneity; df: degrees of freedom; p; p value; I2: I2 test; τ2: tau^2 test; Z: z value.

No differences in lean mass were detected between RTH and RTN (SMD=0.02 [-0.17; 0.21]; Figure 21); subanalyses did not indicate any effects of the studied covariates (Figures 22A, 22B, 22C). No differences in muscle thickness were observed between environmental conditions (SMD=-0.06 [-0.69; 0.57]); there were an insufficient number of studies to carry out subanalyses on this variable (Figure 23).

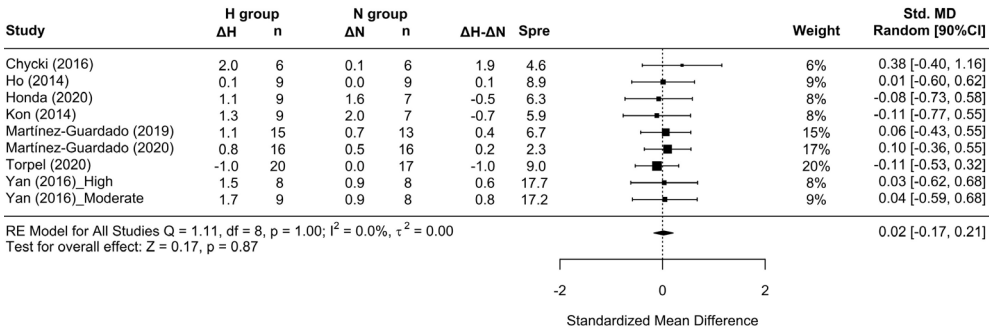


Figure 21 — Forest plot of the standardized mean differences of the total effect of the resistance training program between-conditions (hypoxic [H] group vs. normoxic [N] group) on lean mass. Δ: mean differences between post-pre in H and N or between H-N; n: sample size; Spre: mean baseline standard deviation; Std. MD: standard mean difference; RE: random effect's model; CI; confidence interval; Q: test statistic for the test of heterogeneity; df: degrees of freedom; p; p value; I2: I2 test; τ2: tau^2 test; Z: z value. Yan et al. [332] study provides a group with moderate hypoxia and another with high hypoxia.

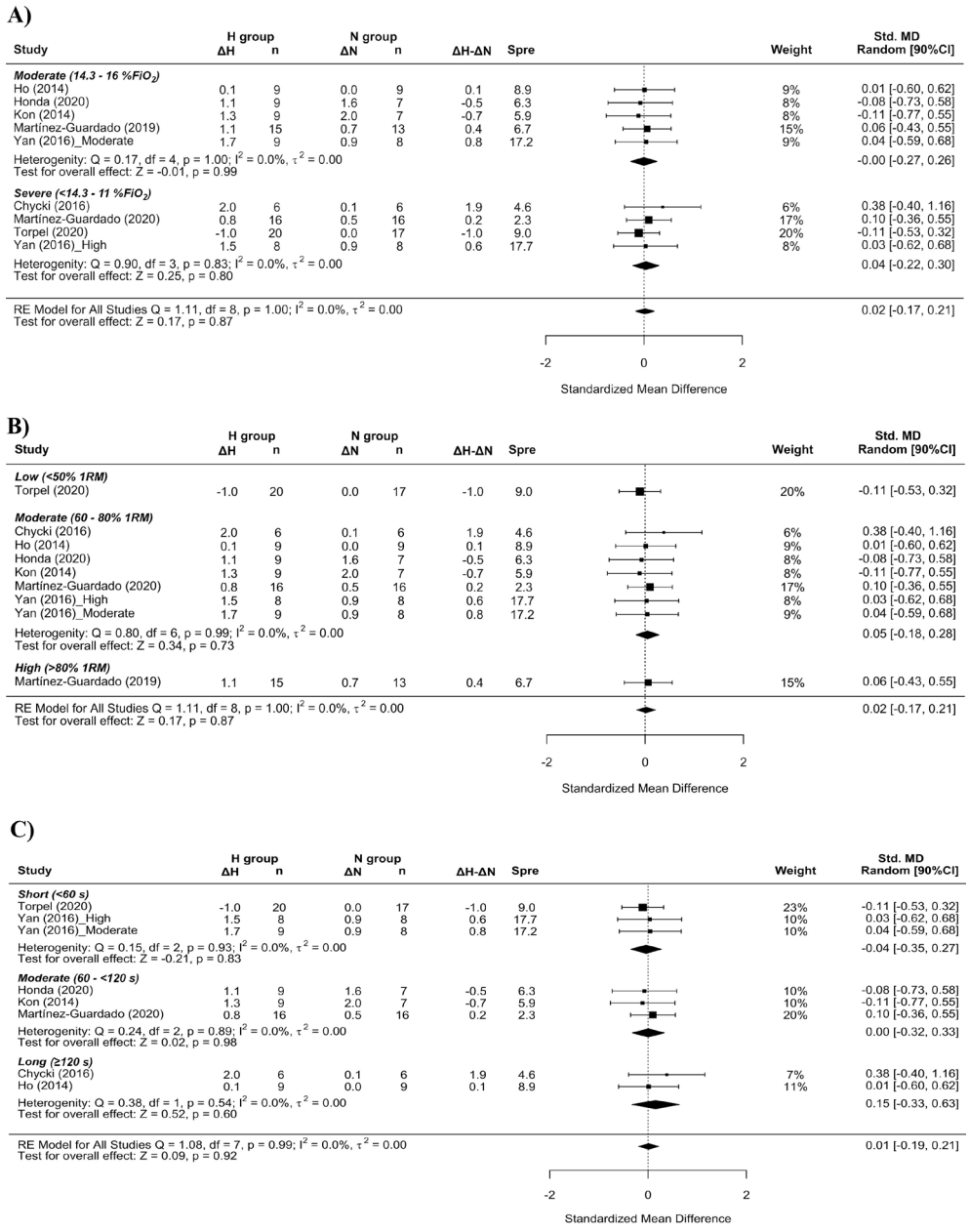


Figure 22 — Forest plot of the standardized mean differences of the resistance training program between-conditions (hypoxic [H] group vs. normoxic [N] group) on lean mass subanalysed by: A) severity of the hypoxia; B) training load; and C) interset rest interval.Δ: mean differences between post-pre in H and N or between H-N; n: sample size; Spre: mean baseline standard deviation; Std.

MD: standard mean difference; RE: random effect's model; CI; confidence interval; F_iO_2: fraction of inspired oxygen; 1RM; 1 repetition maximum; Q: test statistic for the test of heterogeneity; df: degrees of freedom; p; p value; I2: I2 test; τ2: tau² test; Z: z value. Yan et al. [332] study provides a group with moderate hypoxia and another with high hypoxia.

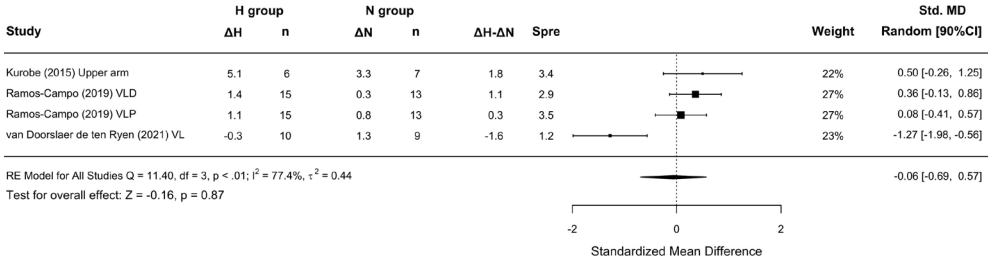


Figure 23 — Forest plot of the standardized mean differences of the total effect of the resistance training program between-conditions (hypoxic [H] group vs. normoxic [N] group) on muscle thickness. Δ: mean differences between post-pre in H and N or between H-N; n: sample size; Spre: mean baseline standard deviation; Std. MD: standard mean difference; Random: random effect's model; CI; confidence interval; Q: test statistic for the test of heterogeneity; df: degrees of freedom; p; p value; I2: I2 test; τ2: tau² test; Z: z value; VL: vastus lateralis; VLD: vastus lateralis distal; VLP: vastus lateralis proximal.

Heterogeneity between studies was found to be low for CSA and lean mass (I^2=0%), and high (I^2 = 77.4%) for muscle thickness.

- *Effect of RTH on strength development*

Twelve studies examined the effect of RTH on strength development. Trivial differences in maximal strength favoring RTH over RTN conditions (SMD=0.13 [-0.0; 0.27]; Figure 24). Subanalysis of the length of the inter-set rest interval showed a medium effect favoring RTH with the use of longer inter-set rest intervals (SMD=0.63 [0.14; 1.12]; Figure 25C). A trivial effect was observed favoring RTH with the use of moderate loads (SMD=0.20 [0.01, 0.40]; Figure 25B) and severe hypoxia (SMD=0.24 [-0.11, 0.58]; Figure 25A). Heterogeneity between studies was found to be low for 1RM between environmental conditions (I^2 = 13.2%).

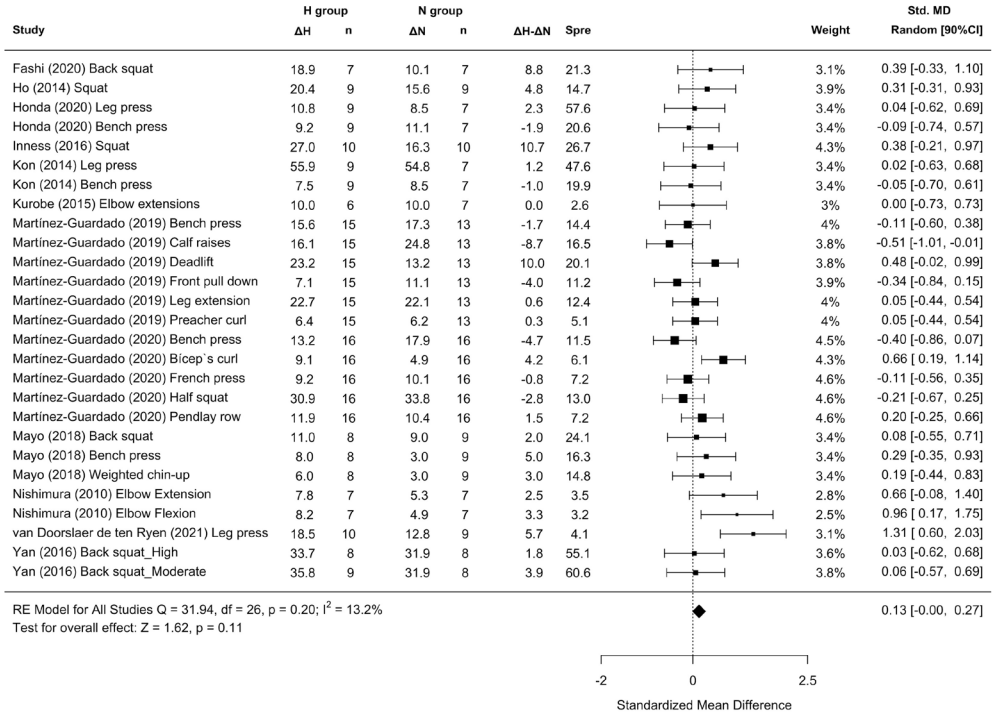
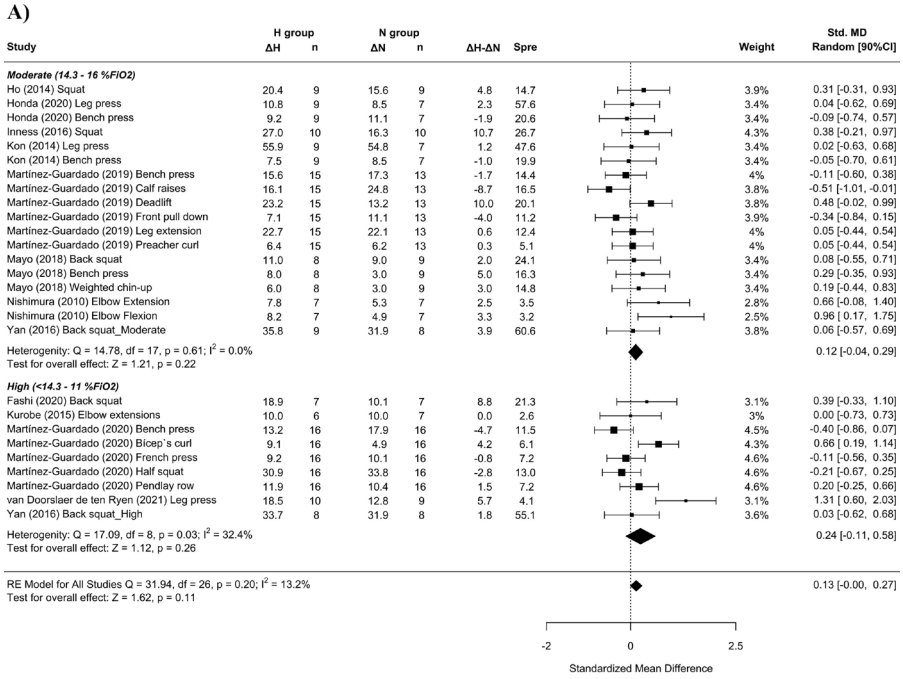
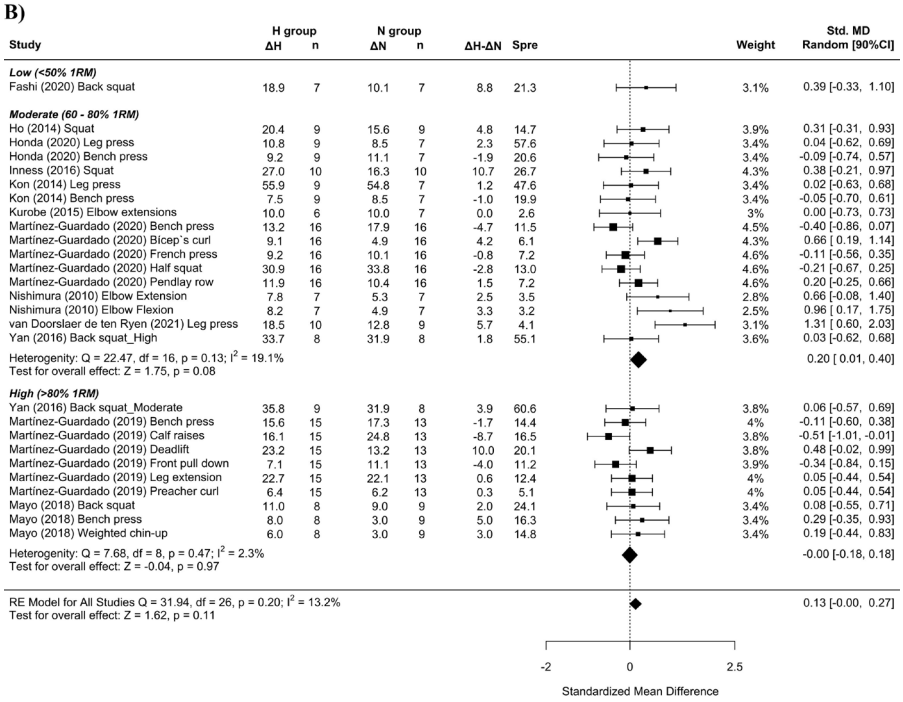


Figure 24 — Forest plot of the standardized mean differences of the total effect of the resistance training program between-conditions (hypoxic [H] group vs. normoxic [N] group) on RM. Δ: mean differences between post-pre in H and N or between H-N; n: sample size; Spre: mean baseline standard deviation; Std. MD: standard mean difference; RE: random effect's model; CI; confidence interval; Q: test statistic for the test of heterogeneity; df: degrees of freedom; p; p value; I2: I2 test; Z: z value. Yan et al.[332] study provides a group with moderate hypoxia and another with high hypoxia.

A)


Study | H group ΔH | n | N group ΔN | n | ΔH-ΔN | Spre | Weight | Std. MD Random [90%CI]
Moderate (14.3 - 16 %FiO2)
Ho (2014) Squat 20.4 9 15.6 9 4.8 14.7 3.9% 0.31 [-0.31, 0.93]
Honda (2020) Leg press 10.8 9 8.5 7 2.3 57.6 3.4% 0.04 [-0.62, 0.69]
Honda (2020) Bench press 9.2 9 11.1 7 -1.9 20.6 3.4% -0.09 [-0.74, 0.57]
Inness (2016) Squat 27.0 10 16.3 10 10.7 26.7 4.3% 0.38 [-0.21, 0.97]
Kon (2014) Leg press 55.9 9 54.8 7 1.2 47.6 3.4% 0.02 [-0.63, 0.68]
Kon (2014) Bench press 7.5 9 8.5 7 -1.0 19.9 3.4% -0.05 [-0.70, 0.61]
Martínez-Guardado (2019) Bench press 15.6 15 17.3 13 -1.7 14.4 4% -0.11 [-0.60, 0.38]
Martínez-Guardado (2019) Calf raises 16.1 15 24.8 13 -8.7 16.5 3.8% -0.51 [-1.01, -0.01]
Martínez-Guardado (2019) Deadlift 23.2 15 13.2 13 10.0 20.1 3.8% 0.48 [-0.02, 0.99]
Martínez-Guardado (2019) Front pull down 7.1 15 11.1 13 -4.0 11.2 3.9% -0.34 [-0.84, 0.15]
Martínez-Guardado (2019) Leg extension 22.7 15 22.1 13 0.6 12.4 4% 0.05 [-0.44, 0.54]
Martínez-Guardado (2019) Preacher curl 6.4 15 6.2 13 0.3 5.1 4% 0.05 [-0.44, 0.54]
Mayo (2018) Back squat 11.0 8 9.0 9 2.0 24.1 3.4% 0.08 [-0.55, 0.71]
Mayo (2018) Bench press 8.0 8 3.0 9 5.0 16.3 3.4% 0.29 [-0.35, 0.93]
Mayo (2018) Weighted chin-up 6.0 8 3.0 9 3.0 14.8 3.4% 0.19 [-0.44, 0.83]
Nishimura (2010) Elbow Extension 7.8 7 5.3 7 2.5 3.5 2.8% 0.66 [-0.08, 1.40]
Nishimura (2010) Elbow Flexion 8.2 7 4.9 7 3.3 3.2 2.5% 0.96 [0.17, 1.75]
Yan (2016) Back squat_Moderate 35.8 9 31.9 8 3.9 60.6 3.8% 0.06 [-0.57, 0.69]
Heterogenity: Q = 14.78, df = 17, p = 0.61; I² = 0.0% 0.12 [-0.04, 0.29]
Test for overall effect: Z = 1.21, p = 0.22
High (<14.3 - 11 %FiO2)
Fashi (2020) Back squat 18.9 7 10.1 7 8.8 21.3 3.1% 0.39 [-0.33, 1.10]
Kurobe (2015) Elbow extensions 10.0 6 10.0 7 0.0 2.6 3% 0.00 [-0.73, 0.73]
Martínez-Guardado (2020) Bench press 13.2 16 17.9 16 -4.7 11.5 4.5% -0.40 [-0.86, 0.07]
Martínez-Guardado (2020) Bicep`s curl 9.1 16 4.9 16 4.2 6.1 4.3% 0.66 [0.19, 1.14]
Martínez-Guardado (2020) French press 9.2 16 10.1 16 -0.8 7.2 4.6% -0.11 [-0.56, 0.35]
Martínez-Guardado (2020) Half squat 30.9 16 33.8 16 -2.8 13.0 4.6% -0.21 [-0.67, 0.25]
Martínez-Guardado (2020) Pendlay row 11.9 16 10.4 16 1.5 7.2 4.6% 0.20 [-0.25, 0.66]
van Doorslaer de ten Ryen (2021) Leg press 18.5 10 12.8 9 5.7 4.1 3.1% 1.31 [0.60, 2.03]
Yan (2016) Back squat_High 33.7 8 31.9 8 1.8 55.1 3.6% 0.03 [-0.62, 0.68]
Heterogenity: Q = 17.09, df = 8, p = 0.03; I² = 32.4% 0.24 [-0.11, 0.58]
Test for overall effect: Z = 1.12, p = 0.26
RE Model for All Studies Q = 31.94, df = 26, p = 0.20; I² = 13.2% 0.13 [-0.00, 0.27]
Test for overall effect: Z = 1.62, p = 0.11
-2
0
2.5
Standardized Mean Difference

B)


Study | H group ΔH | n | N group ΔN | n | ΔH-ΔN | Spre | Weight | Std. MD Random [90%CI]
Low (<50% 1RM)
Fashi (2020) Back squat 18.9 7 10.1 7 8.8 21.3 3.1% 0.39 [-0.33, 1.10]
Moderate (60 - 80% 1RM)
Ho (2014) Squat 20.4 9 15.6 9 4.8 14.7 3.9% 0.31 [-0.31, 0.93]
Honda (2020) Leg press 10.8 9 8.5 7 2.3 57.6 3.4% 0.04 [-0.62, 0.69]
Honda (2020) Bench press 9.2 9 11.1 7 -1.9 20.6 3.4% -0.09 [-0.74, 0.57]
Inness (2016) Squat 27.0 10 16.3 10 10.7 26.7 4.3% 0.38 [-0.21, 0.97]
Kon (2014) Leg press 55.9 9 54.8 7 1.2 47.6 3.4% 0.02 [-0.63, 0.68]
Kon (2014) Bench press 7.5 9 8.5 7 -1.0 19.9 3.4% -0.05 [-0.70, 0.61]
Kurobe (2015) Elbow extensions 10.0 6 10.0 7 0.0 2.6 3% 0.00 [-0.73, 0.73]
Martínez-Guardado (2020) Bench press 13.2 16 17.9 16 -4.7 11.5 4.5% -0.40 [-0.86, 0.07]
Martínez-Guardado (2020) Bicep`s curl 9.1 16 4.9 16 4.2 6.1 4.3% 0.66 [0.19, 1.14]
Martínez-Guardado (2020) French press 9.2 16 10.1 16 -0.8 7.2 4.6% -0.11 [-0.56, 0.35]
Martínez-Guardado (2020) Half squat 30.9 16 33.8 16 -2.8 13.0 4.6% -0.21 [-0.67, 0.25]
Martínez-Guardado (2020) Pendlay row 11.9 16 10.4 16 1.5 7.2 4.6% 0.20 [-0.25, 0.66]
Nishimura (2010) Elbow Extension 7.8 7 5.3 7 2.5 3.5 2.8% 0.66 [-0.08, 1.40]
Nishimura (2010) Elbow Flexion 8.2 7 4.9 7 3.3 3.2 2.5% 0.96 [0.17, 1.75]
van Doorslaer de ten Ryen (2021) Leg press 18.5 10 12.8 9 5.7 4.1 3.1% 1.31 [0.60, 2.03]
Yan (2016) Back squat_High 33.7 8 31.9 8 1.8 55.1 3.6% 0.03 [-0.62, 0.68]
Heterogenity: Q = 22.47, df = 16, p = 0.13; I² = 19.1% 0.20 [0.01, 0.40]
Test for overall effect: Z = 1.75, p = 0.08
High (>80% 1RM)
Yan (2016) Back squat_Moderate 35.8 9 31.9 8 3.9 60.6 3.8% 0.06 [-0.57, 0.69]
Martínez-Guardado (2019) Bench press 15.6 15 17.3 13 -1.7 14.4 4% -0.11 [-0.60, 0.38]
Martínez-Guardado (2019) Calf raises 16.1 15 24.8 13 -8.7 16.5 3.8% -0.51 [-1.01, -0.01]
Martínez-Guardado (2019) Deadlift 23.2 15 13.2 13 10.0 20.1 3.8% 0.48 [-0.02, 0.99]
Martínez-Guardado (2019) Front pull down 7.1 15 11.1 13 -4.0 11.2 3.9% -0.34 [-0.84, 0.15]
Martínez-Guardado (2019) Leg extension 22.7 15 22.1 13 0.6 12.4 4% 0.05 [-0.44, 0.54]
Martínez-Guardado (2019) Preacher curl 6.4 15 6.2 13 0.3 5.1 4% 0.05 [-0.44, 0.54]
Mayo (2018) Back squat 11.0 8 9.0 9 2.0 24.1 3.4% 0.08 [-0.55, 0.71]
Mayo (2018) Bench press 8.0 8 3.0 9 5.0 16.3 3.4% 0.29 [-0.35, 0.93]
Mayo (2018) Weighted chin-up 6.0 8 3.0 9 3.0 14.8 3.4% 0.19 [-0.44, 0.83]
Heterogenity: Q = 7.68, df = 8, p = 0.47; I² = 2.3% -0.00 [-0.18, 0.18]
Test for overall effect: Z = -0.04, p = 0.97
RE Model for All Studies Q = 31.94, df = 26, p = 0.20; I² = 13.2% 0.13 [-0.00, 0.27]
Test for overall effect: Z = 1.62, p = 0.11
-2
0
2.5
Standardized Mean Difference

C)

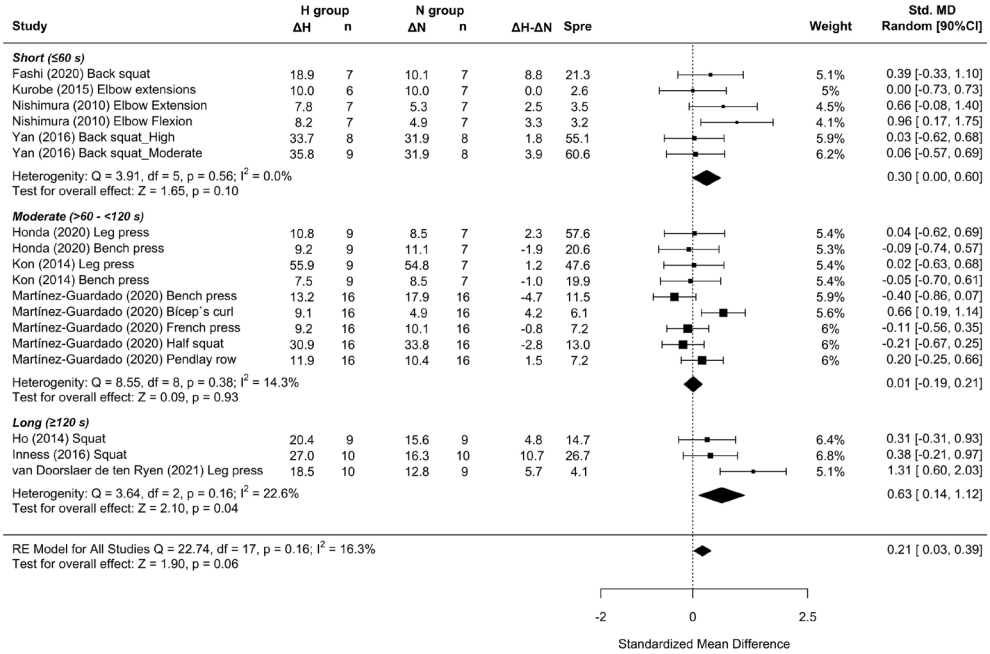


Figure 25 — Forest plot of the standardized mean differences of the resistance training program between-conditions (hypoxic [H] group vs. normoxic [N] group) on RM, subanalysed by: A) severity of the hypoxia; B) training load; and C) interset rest interval. Δ: mean differences between post-pre in H and N or between H-N; n: sample size; Spre: mean baseline standard deviation; Std. MD: standard mean difference; RE: random effect's model; CI; confidence interval; F_iO_2: fraction of inspired oxygen; 1RM; 1 repetition maximum; Q: test statistic for the test of heterogeneity; df: degrees of freedom; p; p value; I2: I2 test; Z: z value. Yan et al.[332] study provides a group with moderate hypoxia and another with high hypoxia.

- *Risk of bias assessment*

The risk of bias/methodological quality of primary studies ranged from medium to high quality (mean quality scores = 5.1/5.3) (Table S4). The risk of bias was unclear for concealment of participant randomization and the blinding of outcome data. All the included studies in this meta-analysis indicated a random sequence generation but did not describe the method used. Egger's test suggested a risk of small study bias for muscle strength outcomes (p=0.001); no risk of small study bias was apparent in regard to muscle hypertrophy outcomes (Egger's test: p>0.05).

DISCUSSION

This systematic review and meta-analysis synthesised and quantified data on studies that directly compared the effects of R_T in hypoxia vs. normoxia on muscle hypertrophy and strength development. Our analysis included 17 studies, almost twice as many as in previous reviews on the topic[343,345,348–352,354]. The inclusion of more recent research (8 additional studies) provided the ability to draw stronger conclusions about the use of RTH for enhancing muscular adaptations.

Similar to previous findings[7–9], a simple pooled analysis without controlling for covariates did not provide compelling support for a potential benefit of RTH vs. RTN on muscle hypertrophy (see Figures 19, 21 & 23) and strength development (see Figure 24). However, subanalyses of data, which considered previously identified potential biases (training load, inter-set rest interval and severity of the hypoxia), suggest a trivial to medium advantage in the use of moderate training loads and short to longer inter-set rest period in R_T at moderate hypoxia on muscular adaptations (see Figures 20, 22 & 25). Other potential confounding factors, such as training status or the type of exercise (one or several exercises, monoarticular or polyarticular, large or small muscle groups, among others), could not be subanalysed due to a lack of sufficient representative data. In this regard, our findings indicate that the use of moderate training loads and longer inter-set rest intervals show a trivial to medium beneficial effect of RTH on strength development compared to the same training protocol under normoxic conditions. However, the inter-set rest period impact on CSA was highly influenced by the fact that only one study employed rest intervals longer than 60s[353]; thus, this finding should be interpreted with some caution. Moreover, the use of moderate loads and hypoxia showed a small beneficial effect on CSA compared to low loads and severe hypoxia. Alternatively, changes in lean mass and muscle thickness changes were similar between normoxia and hypoxia irrespective of covariates.

» Effect of RTH on muscle hypertrophy

R_T is purported to induce muscle hypertrophy through mechanical, metabolic and hormonal processes[21]. The use of multiple sets of moderate

loads and relatively short inter-set rest intervals (60-120 s) between sets has been shown to maximize metabolic stress during R_T[64]. Accordingly, the use of an intermittent hypoxic environment (moderate or severe) during R_T conceivably may elicit a heightened anabolic response compared to normoxic conditions due to the greater accumulation of metabolic byproducts[2,64,293]. Contrarily, evidence shows that chronic exposure (>3 days) to severe hypoxia could contribute to the loss of muscle mass[287]. To date, there is no longitudinal research on the effect of strength training performed in intermittent or chronic hypoxia at moderate terrestrial altitude. All the studies included in this meta-analysis were carried out in normobaric systemic hypoxia.

A pooled analysis of all studies did not show a beneficial effect for RTH on muscle hypertrophy compared to equivalent training in normoxia (SMD<0.17). Subanalysis of studies indicated that the use of shorter inter-set rest intervals with RTH had a small benefit on CSA changes (SMD=0.21 [-0.05; 0.47]). However, longer inter-set rest periods (>120s) also are proposed to extend the capacity to maintain intensities of load and volume during training[2,356], which in turn could supersede any potential benefits of metabolic stress on hypertrophic adaptations. The paucity of studies with moderate and long inter-set rest intervals in this meta-analysis clouds interpretation of the interaction between environmental conditions and rest period length. Further research is needed to elucidate the underlying mechanisms.

The relatively low number of included studies that assessed lean mass[70,345,347–349,352,353,355] and muscle thickness[292,343,350] did not allow us to draw strong conclusions on these outcomes. No appreciable effects were observed between RTH and RTN (SMD=0.02 [CI –0.17, 0.21]) in regard to lean mass. Bioelectrical impedance was used in 3 of the studies[345,348,355], while the other 4 employed dual-energy x-ray absorptiometry[70,347,349,353]; these methods may lack the ability to detect subtle changes in muscle mass[357]. Finally, differences in the composition of exercises in the training protocols (i.e., focused to a specific muscle, body region or full-body) may have also influenced the interpretation of the changes

in lean mass. In particular, 3 of the included studies employed full-body routines[345,348,352], 3 employed 1 exercise for each body region[347,349,353] and only 2 used a single compound leg exercise (back squat)[70,355].

Muscle thickness was the least-used method for estimating changes in muscle size in RTH. Among the 3 available studies on this outcome, only one [350] found a detrimental effect of RTH under severe hypoxia in untrained participants (13.5% FiO_2). Conversely, Kurobe et al. [292] and Ramos-Campo et al. [343] reported no significant changes between conditions under severe and moderate hypoxia in untrained and trained populations, respectively. Additionally, differences in the training loads and inter-set rest intervals used among studies compromised the statistical power of the meta-analysis and thus made it difficult to draw strong conclusions about the effect of RTH on muscle thickness.

» Effect of RTH on strength development

The maximum muscular strength was evaluated via the pre-post study change in 1RM. Pooled analysis of all studies did not indicate that RTH increased maximal strength to a greater magnitude than the same training under normoxia; although the direction of the interaction favored RTH, the point estimate indicated minimal benefits on this outcome (SMD=0.13 [-0.00; 0.27]). Conversely, subgroup analysis of the data identified long inter-set rest intervals (SMD=0.63 [0.14; 1.12]) and moderate loads (SMD=0.20 [0.01; 0.40]) as positive modulators of strength development in RTH, regardless of the severity of hypoxia. Hypoxic conditions varied considerably between studies (ranging from 12 to 16% FiO_2) and did not seem to meaningfully influence 1RM outcomes; this finding is in opposition with that of hypertrophy, where moderate hypoxia showed a favorable effect on CSA increases. The underlying mechanisms for the observed discrepancy are not readily apparent and warrant future investigation.

The potential of RTH to improve muscle strength is thought to be largely mediated by hypertrophic adaptations[290]. Hypoxia-mediated neural adaptations, generally linked to the use of heavy loads (>85% 1RM)[76], remain

poorly elucidated. Nevertheless, as previously mentioned, the observed benefit of moderate loads during hypoxic training could be limited by inconsistencies between experimental designs and participant training level. In this regard, differences in the frequency of weekly sessions and duration of the training protocols confound the ability to draw strong inferences, since neural alterations start during the early phase of training in untrained subjects[22,346]. Finally, it could be argued that hypoxia per se may not confer a favorable environment for neural adaptations in strength training compared to RTN. This hypothesis should be further investigated in future research.

The body of research that included training protocols specific to muscular strength improvements (e.g., higher loads with longer inter-set rests) did not show a benefit to conditions of systemic hypoxia[293]. Only 1 of the 3 included studies that employed long inter-set rest intervals[350] showed a clear benefit of hypoxia for strength development (Figure 8.C), which only would partially support this beneficial effect. Indeed, recovery periods ≥120 s seem to mitigate any additive benefit from the hypoxic stimulus[293], while shorter inter-set rest periods could entail more challenging metabolic conditions for muscle development[358]. In contrast, our results revealed that longer rest periods produced moderate gains in 1RM after RTH compared to RTN (SMD=0.63 [0.14; 1.12]). This discrepancy may be due to the use of untrained samples in 2 of the studies [350,355]. Grgic et al.[242] proposed the use of inter-set rest intervals >120 s in trained subjects and from <60 to <120s in untrained individuals to maximize gains in muscular strength in normoxia. Hence, the results of our meta-analysis could be influenced by the fact we were not able to subanalyse data based on the participants' training status.

Section II. Preliminary studies on acute exposure to hypobaric hypoxia.

Study 2. Altitude-induced effects on muscular metabolic stress and hypertrophy-related factors after a resistance training session.

RESULTS

All participants reached muscular failure between 8 to 12 repetitions during both training sessions. Training load, number of repetitions and total-volume load during the 3 sets of the 3 free barbell exercises in both training conditions are showed in Table 8. Slight differences in the load or in repetitions to failure were observed due to the load adjustment on successive sets to ensure maintenance of the target repetition range. Small to moderate mean variations of less than 1 repetition per set were observed between conditions in both the flat barbell press and the barbell back squat exercises, while the absolute load showed a non-significant mean increase from 0.24 to 1.54 kg respectively. H and N training sessions showed similar RPE values 30 minutes after the end of the training (8.5 ±1.4 vs. 8.6±0.8 respectively for N and H; p=0.603; 95%IC [-1.00; 0.5]; ES=0.14). Maximal blood lactate presented no differences between N and H (16.08 ± 3.73 vs. 15.52 ± 2.66 mMol·l^{-1} in N and H; p= 0.322; 95%IC [-1.73; 0.62]; ES=0.17) and the lactate recovery curve was similar between conditions (Figure 26).

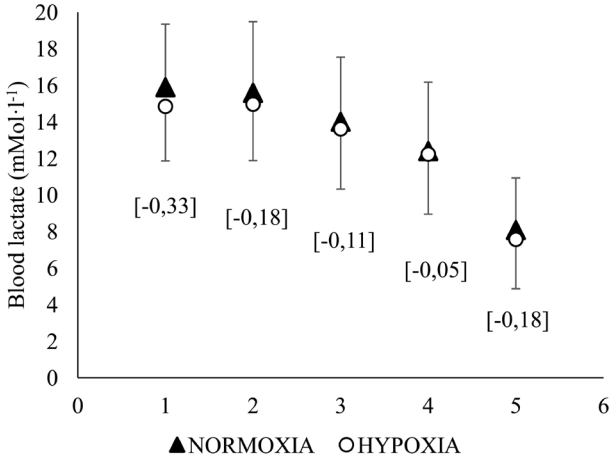


Figure 26 — Blood lactate concentration throughout the recovery period in normoxia and hypoxia condition. No significant differences between conditions; [ES]: effect size calculated as (H mean-N mean)/ (pooled standard deviation).

Table 8 — Training load and number of repetitions accumulated during the three training sets in both training conditions.

	Training load (Kg)				Rep x set				Total volumen-load (Kg)	
	N	H	Δ (Kg)	ES	P [95% CI]	N	H	Δ (Kg)	ES	P [95% CI]	N	H
Bench press	66.3 ± 11.4	66.5 ± 11.4	0.2	0.02	0.557 [−0.64;1.12]	10.1 ± 1.0	9.7 ± 0.9	0.4	0.38	0.009 [−0.6; −0.11]	1997.4 ± 322.2	1936.9 ± 332.8*
Military press	36.0 ± 4.6	35.0 ± 4.0	-1.0	-0.24	0.125 [−2.38;0.33]	8.6 ± 0.6	8.5 ± 0.5	-0.1	-0.14	0.661 [−0.45; 0.29]	922.2 ± 123.2	891.8 ± 125.3
Back squat	74.3 ± 12.7	75.9 ± 15.0	1.5	0.11	0.219 [−1.78; −0.11]	11.0 ± 0.7	10.1 ± 1.2	0.9	0.99	0.029 [−1.78; −0.11]	2460.8 ± 463.6	2261.9 ± 331.4*

N: normoxic condition; H: altitude condition; 10RM: 10 repetition maximum; Rep × set: mean repetitions completed per set; Change: mean percentage of change between conditions in the load or repetition sets; Δ: absolute mean group difference between conditions in the load or repetitions sets; ES: effect size [calculated as (H mean-N mean) ÷ (pooled standard deviation)]; P: p value; 95% IC: 95% interval of confidence. *p < 0.05: Significant difference between N and H conditions in the Total Volumen-Load variable.

The results for Ca^{2+}, CO_2-L and Pi values under basal conditions and through the recovery curve between conditions are displayed in Figure 27. Significant increases in Ca^{2+} ($P<0.001$) and reductions in Pi and CO_2-L were observed after the R_I exercise versus the basal condition in H ($p<0.001$) and N ($p<0.05$). The results of the repeated measures ANOVA showed a main effect of time in all these variables due to a change in the values each 5 minutes throughout the recovery period ($p<0.05$). The Pi and CO_2-L displayed an environmental condition main effect ($F = 12.22$, $p = 0.007$, $\eta^2_G = 0.58$ and $F = 14.42$, $p = 0.004$, $\eta^2_G = 0.59$) due to the lower values across the recovery time in the hypoxia condition (2.89 ± 0.64 vs. 2.23 ± 0.60 $mg{\cdot}dl^{-1}$; 21.11 ± 1.46 vs. 16.19 ± 1.61 $mmol{\cdot}l^{-1}$, for N and H respectively). No recovery time × environmental condition effect was observed in ions ($F = 0.689$, $p = 0.515$, $\eta^2_G = 0.06$; $F = 2.03$, $p = 0.133$, $\eta^2_G = 0.18$; $F = 0.711$, $p = 0.553$, $\eta^2_G = 0.06$ respectively for Ca^{2+}, Pi and CO_2-L).

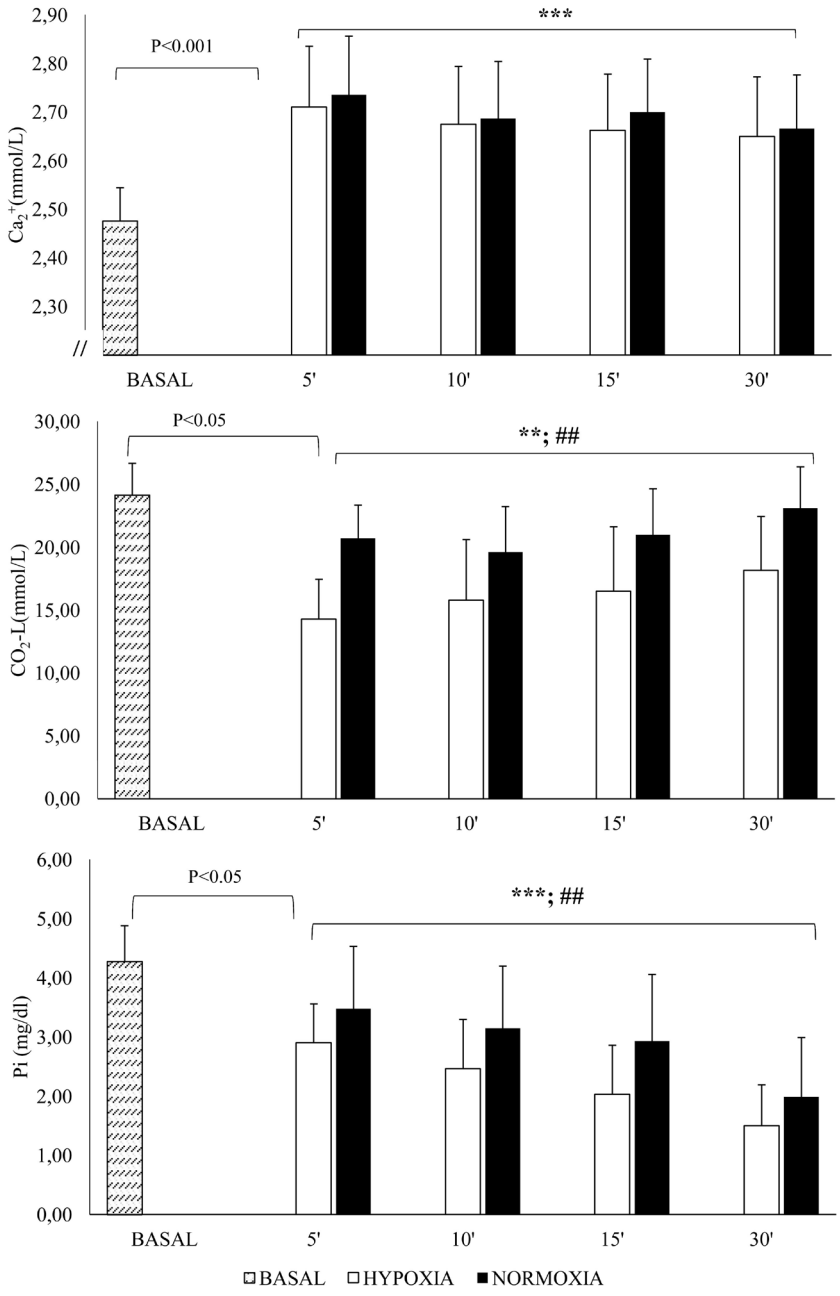


Figure 27 — Comparison of calcium (Ca^{2+}), liquid carbon dioxide (CO_2-L) and inorganic phosphorus (Pi) values in basal and between altitude condition through the recovery period. * time effect (* p<0.05; **p<0.01; ***p<0.001; # altitude effect (# p<0.05; ## p<0.01; ### p<0.001; ¥ interaction time x altitude condition (¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.

No differences between the basal condition and at 5 minutes of the recovery were observed in testosterone. However, there was a main effect of time (F = 14.37, p = 0.001, η^2_G = 0.59) due to a progressive reduction in testosterone throughout the recovery. This behavior was similar in both altitude conditions (F = 2.50, p=0.145, η^2_G = 0.20) (Figure 28). No recovery time or environmental condition main effect was observed (F = 0.38, p=0.603, η^2_G = 0.04; F = 1.02, p=0.339, η^2_G = 0.10 respectively) for GH and an increase by more than 82 times after the training session compared to basal values was observed. No interaction recovery time × environmental condition effect was observed in any of the studied hormones (F = 2.03, p= 0.131, η^2_G = 0.17; F = 0.39, p= 0.654, η^2_G = 0.04 respectively for testosterone and GH) (Figure 28).

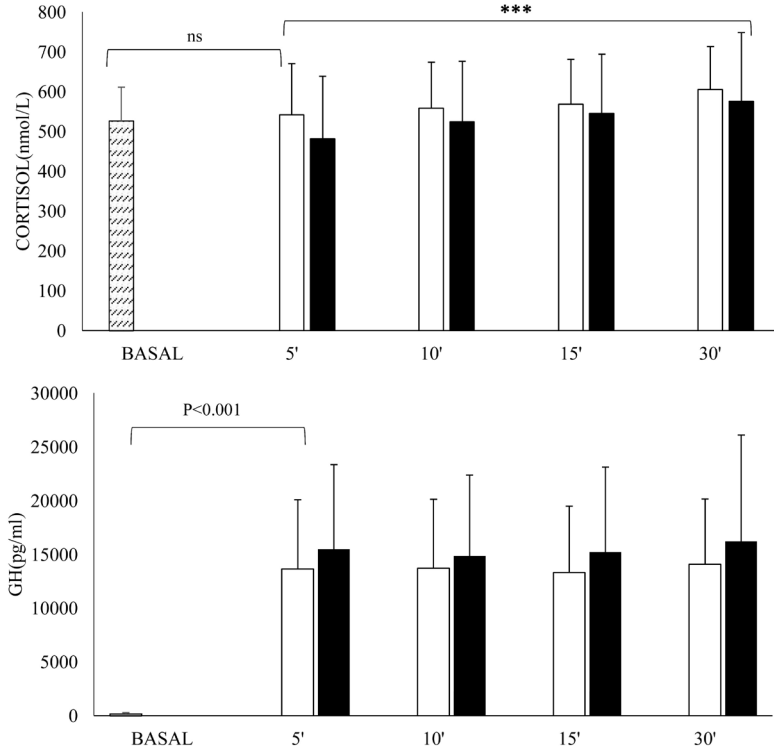


Figure 28 — Comparison of testosterone and hormone of growth (GH) values in basal and between altitude condition through the recovery period. * time effect (* p<0.05; **p<0.01; ***p<0.001; # altitude effect (# p<0.05; ## p<0.01; ### p<0.001; ¥ interaction time x altitude condition (¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.

DISCUSSION

The aim of this study was to investigate the effect of acute moderate altitude on the metabolic stress and associated responses induced by a hypertrophy-oriented R_T session. Contrary as expected, the results did not display differences in RPE, maximal blood lactate, Ca^{2+} and anabolic hormones (testosterone and GH) responses when a similar R_T (3 sets x 10RM, 2 min rest) was performed in both environmental conditions. Even post-exercise Pi displayed lower values in H when compared to N. Thus, the results of this study do not support the theory that R_T combined with acute terrestrial hypoxia enhances factors linked to muscle growth.

Based on the possibility of additional high-threshold motor units' recruitment due to the augmented anaerobic metabolism in hypoxic conditions, an increase in performance during R_T at moderate altitude could be expected[64,290,293,295]. In the present study, although some differences were detected in the training load and in the number of repetitions to failure between N and H, the absolute values amounted to less than 1 repetition or 1.6 kg in exercises such as bench press and back squat. The interpretation of training load magnitude must jointly consider the modulation of the load and the number of repetitions to failure accumulated. In back squat and bench press exercises, a mean reduction of 0.36 and of 0.95 repetitions per set was observed in H, consistent with the small but nonsignificant increase in the workload (between 0.2 [∼0.5%] and 1.5 kg [∼2%] respectively for each exercise). In addition, all participants reached muscular failure between 8 and 12 repetitions and kept a constant cadence, which allows direct comparison of the training response in both conditions (see Table 8).

A mean s-RPE value of ∼ 8.5 was observed in this study, consistent with previous studies that employed a hypertrophy-oriented training routine (6-12 RM; 8-10 RPE)[359]. No differences in s-RPE were detected between conditions. This result is consistent with Scott et al.[360] who reported no changes in RPE after a traditional heavy R_T session between moderate (FiO_2 =16%) and severe (FiO_2 =13%) hypoxia compared to N. Conversely, significant increments in RPE between high (FiO_2=

13%) and moderate (FiO_2= 16%) hypoxia and N conditions have been observed after a high-intensity resistance circuit[361] or several all-out sets of continuous jumps[362]. The way that RPE was collected (immediately after the training bout vs. after each set vs. 30 min after the end of the training bout [s-RPE]), the exercise protocols (jumping vs. traditional vs. high-intensity resistant circuit) and the implicit differences in the loads used (body weight vs. moderate vs. heavy load). could explain the contradictory RPE response after R_T under hypoxic conditions. Some studies hypothesize that recovery times longer than 90 s could compromise hypoxic effects and thus mitigate the potential anabolic impact of metabolic stress[292,295,355]. Therefore, the somewhat longer inter-set rest intervals employed in this study (2 min) could favor PCr re-phosphorylation[363,364] and also the degree to which metabolic byproducts are removed from the muscle before the ensuing set[293]. This is compatible with the absence of change in blood lactate and circulating Ca^{2+} between conditions in our results. Some studies have reported similar blood lactate results at moderate hypoxia after a high intensity R_T circuit[71], high load traditional R_T[297], explosive strength session[362] or high-intensity intermittent exercise[365]. While others found higher blood lactate levels after low to high intensity R_T at moderate[295,360] and severe hypoxia R_T[67–69,361]. The participants training status and R_T protocol design (i.e., muscles involved, number of exercises, intensity and volume) could affect the magnitude of this response, since our results reported maximal blood lactate above that reached in all the aforementioned studies (~ 16 vs. 5 to 8 mmol·l^{-1}). However, the lower CO_2-L levels detected in H, also shown in other studies[361], clearly indicate a higher muscle buffering response at this condition, which could reduce the net blood lactate measured in H. Moreover, lactate enters the bloodstream via concentration gradient differences and by the use of carbon monoxide transporters[366,367] reducing the effectiveness of both mechanisms while lactate concentration increases. A possible explanation for the similar maximal lactate values observed in both sessions could be the greater buffering activity and the slow lactate release from muscle in H, that limits the ability to differentiate an increase

in glycolytic pathway in H[368]. Thus, lactate levels may have been higher in H, but this hypothesis requires further study.

As noted above, we observed an unexpected reduction in Pi after the R_T in H (∼20% minor in H along the 30 min of recovery) compared to N. The maximal aerobic capacity of single muscles is more limited by peripheral factors than central ones during ATP production under no O_2 limitations [364]. Thus, throughout the initial hours of exposure to moderate hypoxia, there is an increase in ventilation, submaximal heart rate and cardiac output [369], which act as compensatory mechanisms to increase muscle buffering capacity, O_2 availably and PCr restauration rate [361]. Moreover, the shift to the right of the oxyhemoglobin curve (O_2-Hb) during exercise in H (Bohr Effect, the increase in 2,3-bisphosphoglycerate and pH elevation), improves the oxygen release in active muscles [370]. Taken together, the acute improvements in cardiac function and the O_2-Hb curve, combined with the non-predominant aerobic pathway during the R_T methodology assessed, could favor recovery between sets, accelerating the regeneration of ATP and PCr in acute H. According with the Pi results the Ca^{2+} levels did not change after the training session [358]. Ramos-Campos et al. [361]. also found similar serum Ca^{2+} during R_T at normoxia, moderate or severe acute hypoxia. Further research is needed to establish the role of the oxygen availability on ATP and PCr resynthesis during intervening rest periods.

The results obtained did not reflect a significant altitude effect on testosterone and GH after the training sessions. The GH levels seems to be mediated by blood lactate and H^+ [69,293]. Our results showed similar elevated maximal lactate and GH levels in both conditions. Indeed, increases in blood lactate and GH after low to moderate R_T sessions have been detected in other studies under acute hypoxic conditions [67–70]. When analyzing testosterone, several studies have reported increases after a hypertrophy-oriented R_T session[112,115]. But, similar to our results, other researchers have failed to detect differences in post-exercise testosterone secretion under N and H conditions[67,69,70,292], finding the maximal value occurs immediately after the R_T session[70]. A relationship between

testosterone response and metabolic stress has not been well-established[371] and other factors related to the training protocol (i.e. muscle mass implicated, intensity or volume) could be more influential [372,373]. It therefore can be speculated that the acute serum testosterone response to R_T may not be significantly affected by hypoxia [67,70]. We would note that although chronic anabolic hormonal levels have been considered as key factors for skeletal muscle development, the role of the acute endocrine response to exercise on muscle growth in this regard remains dubious [374,375]. Thus, additional research is needed to elucidate the role of these hormones for hypertrophy in R_T.

Study 3. Hormonal and Inflammatory Responses to Hypertrophy-Oriented Resistance Training at Acute Moderate Altitude.

RESULTS

Cytokine results are displayed in Figure 29. The kit used for these analyses proved not to be sensitive enough to determine interleukin changes in healthy trained athletes (i.e., the threshold for a detected value was too high so that changes that may have occurred went undetected). Thus, we employed a non-parametric dichotomous statistical analysis (McNemar test) on these measures to compare detected vs. non-detected values (i.e., compare the change from 0 to 1 or 1 to 0 from pre- to post-exercise in both environmental conditions). The McNemar test did not show statistical significance at any condition or recovery time in the three cytokines analyzed. However, large increases were observed in the detected IL-6 values after the training session at both environmental conditions (ES=1.68 and ES=1.77 for N and H, respectively). The highest detected IL-6 value was reached earlier in N (min 15) compared to the H condition (min 30) (mean ± SEM: min 15 [7.55±3.21 vs. 5.44±2.68 pg/ml]; min 30 [2.93±0.01 vs. 10.10±0.22 pg/ml] for N and H, respectively) (Figure 29A). Moderate to large increases in IL-10 also were observed after R_T in N (ES=0.80, IC [-0.39;1.98]) and H (ES=1,52, IC [0.17;2.88]) (Figure 29B). The magnitude of the change displayed larger peaks values in IL-10 throughout the 30 min of recovery in H (1.02±0.19 vs.

2.31±0.44 pg/ml, for N vs. H, respectively maximum values). The TNFα reached the maximal value in min 15 of the recovery in both conditions although moderate increases favoring H were also depicted (ES=1.01, IC [-1.18;3.21] pg/ml) (Figure 29C).

Figure 30 shows the hormonal results. Cortisol (C) and growth hormone (GH) significantly increased after the training session (Fig 30A and 30B). Although no statistical differences between environmental conditions were found in the three hormones studied, testosterone displayed a tendency to be reduced post-exercise in H compared to N (7.52±2.25, 6.87±2.09 ng/ml for N and H, respectively; p=0.06) (Figure 30C).

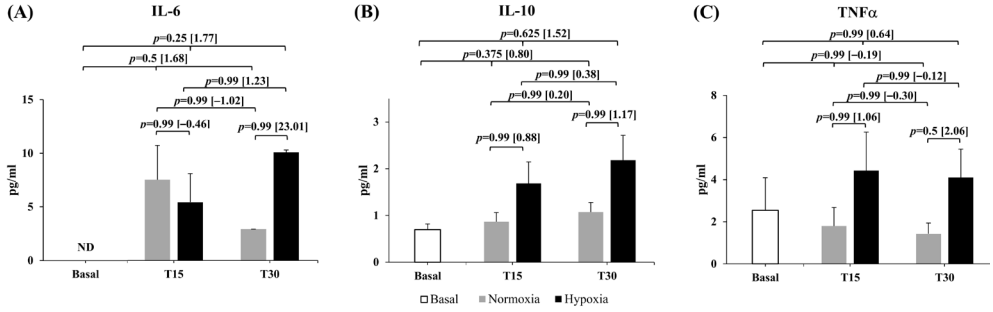


Figure 29 — Analysis of the distribution of detected vs. non-detected values of IL-6 (A), IL-10 (B) and TNF-alpha (C) in N and H through the 30 min of recovery period. Inference analysis for detected cytokines. Blood samples were taken at rest and 15 minutes (T15) and 30 minutes (T30) after exercise in both N and H conditions. Mean and SEM was represented only for detected signal. p-value (p) and effect size [ES] are represented for each variable. ES was expressed as H-N or post-pre divided by pooled standard deviation; ND: non-detected.

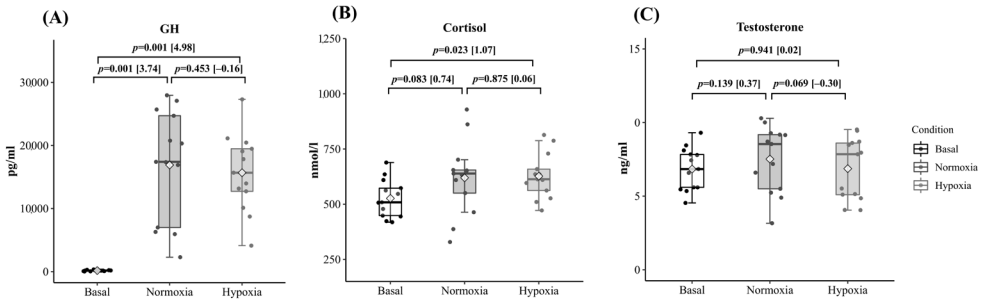


Figure 30 — Comparison of the training session effect on maximum post-exercise circulating growth hormone (GH-A), cortisol (B) and testosterone (C) in N and H conditions. Data are presented as median, 25th and 75th percentile and maximum and minimum values. The point clouds are also included. The P values are displayed as differences between basal and the corresponding training session. Effect size [ES] is calculated as the mean change (H-N) or (post-pre) divided by the pooled standard deviation.

Neither environmental condition affected the circulating level of miR-378 after R_T (p=0.78, p=0.54 for N and H, respectively). Trivial non-significant increases were achieved after the 30-minute recovery period in both conditions (1.75 ± 1.58 vs. 1.88 ± 1.32 arbitrary units in N and H; p=0.854; 95%IC [-1.33; 1.59]; ES=0.09) (Figure 31).

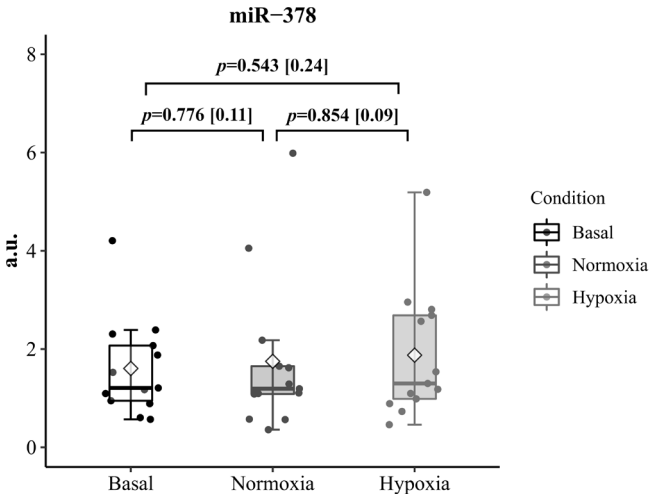


Figure 31 — Comparison of the training session effect on maximum post-exercise circulating miR-378 in N and H conditions. Data are presented as median, 25th and 75th percentile and maximum and minimum values. The point clouds are also included. The P values are displayed as differences between basal and the corresponding training session. Effect size [ES] is calculated as the mean change (H-N) or (post-pre) divided by the pooled standard deviation.

The correlation analysis displayed only a moderate association between miR-378 with GH and C in N and H (GH: r=0.654; p=0.03 and r=-0.591; p=0.05; C: r=0.61; p=0.03 and r=0.75; p=0.005 respectively) (Table 9). However, a large effect of the environment on the relationship with miR-378 was observed with GH and testosterone. The logistic regression between cytokines and miR-378 did not show a significant relationship in N (IL-6: R^2=0.20; p=0.637; IL-10: R^2=0.40; p=0.116 and TNFα: R^2=0.21; p=0.174) and H (IL-6: R^2=0.11; p=0.279; IL-10: R^2=0.01; p=0.839; and TNFα: R^2=0.19; p=0.241).

Table 9 — Relationship between the miRNA-378 response and the peak value of the hormone's serum concentrations in both altitude condition.

	miR-378				**N vs H**		
	N		**H**				
	r	**p**	***r***	**p**	**Z**	**p [F>2.69] =0.05 p [F>4.16] =0.01 [%95 IC]**	**Q**
GH (pg·ml^{-1})	**0.654**	**0.026**	-0.591	0.051	-4.621	**p=0.01** [-2.081; -0.841]	1.461 Large
Testosterone (ng·ml^{-1})	0.462	0.128	-0.456	0.133	-3.139	**p=0.05** [-1.613; -0.373]	0.993 Large
Cortisol (nmol·l^{-1})	**0.606**	**0.035**	**0.747**	**0.005**	0.833	- [-0.356; 0.883]	0.263 Small

r: Pearson correlation coefficient; p: p-value; Z: value of the statistic in the Fisher's Z-transformed *r* coefficient comparison; F: value of Fisher's F distribution only for p=0.05 or p=0.01. Non-significant values are not expressed; Q: Cohen´s q effect size; N: normoxia; H: hypoxia; miR: microRNA.

DISCUSSION

The aim of this study was to investigate the influence of the acute ascent to a moderate hypoxic environment on the response induced by a hypertrophy-oriented R_T session on inflammatory, hormonal and miR-378 markers. Exercise increased maximal values in GH and C, while testosterone and miR-378 remained similar to those observed under basal conditions. The observed post- R_T response on inflammatory markers suggests an intensification of these cytokines in N and H; however, differences did not rise to statistical significance due to the lack of sensitivity of the analysis kit, limiting

our ability to draw a strong conclusion on this outcome The magnitude of the change in the detected values also seems to indicate a hypoxia-related effect on IL-6, IL-10 and TNFα release into the bloodstream throughout the recovery period. Moreover, IL-6 displayed a larger, albeit delayed, increase in H compared to N. Despite the lack of changes in circulating levels of miR-378, the moderate association with GH and C agrees with the research hypothesis and may indirectly indicate a relationship with the expression of other target genes involved in muscle hypertrophy [64].

The results displayed a rapid R_1-induced inflammatory response, which has been attributed to an acute potentiation of myogenesis through the circulating TNFα and IL-6 [226]. The magnitude inference analysis of the results showed a delay in the increase of IL-6 in H compared to N after exercise. IL-6 is both a pro- and anti-inflammatory cytokine 150, released in an exponential fashion into the blood stream in response to damaging exercise and depletion of muscle glycogen stores [376]. It can be hypothesized that the greater stabilization in pH observed in N in several studies [361,377] allows greater glucose resynthesis [378], which may explain the early increase in IL-6 in this condition and the limited TNFα production, highly dependent on O_2 availability. Conversely, it is known that during exercise in H, an increase in reactive oxygen species (ROS) associated with the rise in the expression of hypoxia-inducible factor-1 (HIF-1) [379], in conjunction with the high buffering activity that limits the ability to resynthesize glucose [377], could help to explain the progressive and delayed increase in IL-6 through TNFα overexpression [380,381]. An increase in circulating IL-6 potentially enhances anabolic signaling via STAT3, which is required for the increase in the myogenic regulatory factors (such as MyoD), as well as by inhibiting the apoptotic pathways of TNFα through SOCS1 expression (Figure 1) 150,382,383. As has been observed in other studies [226], the net IL-6 production is intensified in H. The progressively larger increases in IL-10 release throughout the recovery period with respect to the N values achieved could also emphasize this response [384,385]. Moreover, although a positive association between IL-10 and C levels was expected, it was not

observed, probably due to the aforementioned limitation in the IL-analysis procedure. The present results failed to clearly show that the same R_T session performed under H conditions enhances the hormonal, inflammatory and circulating miR-378 responses compared to normoxia. However, it is possible that hypoxia may prolong the duration of time that various cytokines circulate post-exercise compared to N, which potentially could enhance the hypertrophic response.

R_T produced large post-exercise increases in circulating GH and C, although altitude did not statistically affect the magnitude of their peak values. This may be explained by the similar metabolic stress response (i.e., maximal blood lactate and serum Ca^{2+}) observed for both environmental conditions [377]. Consistent with this hypothesis, C dynamics reflect the global stress and metabolic requirements [115]. GH release also seems to be mediated by blood lactate and H^+ levels [69,360]. However, despite the general increase in lactate production during hypoxic R_T described in the literature [68,69,295], some studies have failed to observe statistical differences in peak lactate [377] and GH levels after R_T under hypoxic conditions compared to N [353]. The type of hypoxia alters the physiological response of systems, such as the ventilatory system, affecting the behavior of variables sensitive to changes in the ventilatory pattern 11. Therefore, the hypocapnic response throughout the initial hours of exposure to terrestrial hypoxia found in other studies [71,377] seems to increase the pre-exercise pH and potentially helps to explain the physiological reduction of GH release during acute exercise at terrestrial H vs. at normobaric hypoxia [138].

The results do not reveal a significant change in circulating testosterone after the acute hypertrophic strength session and hypoxia does not seem to affect it [70,377]. Although H revealed a small decrement in the peak circulating post-exercise testosterone response compared to N, the absence of differences in this hormone with respect to basal conditions when contrasted with the changes in GH and C blood values, highlights the complexity of the acute hormonal response to strength exercise.

In addition to the inflammatory and hormonal response, the effects of hypoxia on miR-378, which is linked to protein synthesis regulation,

potentially could elucidate the miRNA relationship with other muscle building agents in trained individuals [227]. The overexpression of miR-378 has an inhibitory effect on its target gene expression that, in some way, regulates the processes that lead to muscle hypertrophy by allowing the MyoD factor to be expressed, as also IL-6 does [226]. Contrary to our expectations, circulating miR-378 levels showed similar values between basal conditions and after acute R_T in N (p=0.776; 95% IC [-1.236,0.945]) and H (p=0.543; IC95% [-1.214; 0.067]) and did not display any relationship with IL-6. Under N conditions, increases in circulating miR-378, miR-21 and miR-940 are described immediately after acute aerobic exercise in chronic heart failure patients [386]. To our knowledge, only one study [227] has investigated this outcome in healthy individuals during R_T although, unlike in our study, the researchers measured the expression of miR-378 and other miRNAs from muscle biopsies rather than serum samples. Davidsen et al. [227] observed elevations in miR-378 expression and reported a positive relationship between the abundance of this miRNA and muscle mass gains after a 12-week R_T program. miR-378 displayed a down-regulation in those classified as low responders for lean mass gains (linked to a reduced miRNA profile for IGF-1), whereas high responders exhibited no changes from baseline to post-exercise. The fact that miR-378 is not expressed specifically in muscle tissue could explain its relevance as a biomarker of exercise adaptations in clinical populations [386] and with sedentary individuals [227], while limiting its applicability to trained individuals. Thus, the variability between subjects could corroborate the presence of responding and non-responding participants. This hypothesis warrants further investigation.

Hypoxia seems to produce transient increases in IGF-1 mRNA after a R_T [226]. Currently, 3 different IGF1 isoforms are described, all of them expressed in muscle tissue. In particular, the IGF1-Ec isoform, also known as mechano growth factor (MGF), is expressed immediately post-exercise to facilitate the repair of local muscle damage through a signaling pathway that includes mTOR (PI3K-Akt-mTOR) and other calcium-dependent pathways. The link between GH and IGF1

is also documented in the literature 34. Our study did not measure IGF1, but the relationship between GH and miR-378 observed in N could indirectly indicate the activation of the protein synthesis pathway linked to the miR-378 response. The association observed between GH and miR-378 is consistent with our research hypothesis, although the weakness of the association, as well as the absence of a relationship with other markers related to muscle growth (such as testosterone or IL-6) raises skepticism as to potential conclusions. The interaction displayed between H and GH [138], and also indirectly with testosterone (Table 9), could conceivably help to explain the H effect on the association between miR-378 and these hormones. However, the absence of activation of the mTOR pathway after an isolated R_T session [294] at acute hypoxia may question the influence of the IGF1, GH and mR-378 activation pathway in the early adaptations of skeletal muscle to resistance exercise. Longer duration studies are required to clarify the role of these mediators in muscle hypertrophy in response to R_T in hypoxia.

Section III. Acute effect and comparison between types of hypoxia.

Study 4. Inter-set rest configuration effect on acute physiological and performance-related responses to a resistance training session in terrestrial vs. simulated hypoxia.

RESULTS

» Resistance training session

Table 10 displays the mean total volume-load accumulated during the 3 sets of the 2 free barbell exercises across conditions. The adjusted between-group effects showed no meaningful differences in volume-load between both types of hypoxia at each of the inter-set rest intervals in the 2 analyzed exercises (adjusted between-group effect from -7.64 to 51.75 kg [90% CIs from -135 to 238.53 kg] and from -43.05 to -15.55 kg [90% CIs from -110.03 to 51.21 kg], respectively for 60 and 120 s inter-set rest intervals). However, trivial to moderate increases in the total volume-load were achieved at longer inter-set rest periods in the bench press at HH (5.9%, ES=0.35, p=0.027).

» Cardiovascular, metabolic and perceptual responses

Heart rate, blood lactate and RPE-30 responses are presented in Table 11. The results showed moderately lower mean work and rest-HR values with 120 s inter-set rest periods at normoxia (ES: from 1.01 to 1.08) and both types of hypoxia (ES: from 0.58 to 0.92). A similar work-HR response was observed between HH and NH conditions. However, we detected a lower mean rest-HR in NH during both inter-set rest intervals than in HH (adjusted between-group effect of 13.56 bpm [90% CIs: -0.85, 27.97 bpm] and 16.12 bpm [90% CIs: -1.73, 33.98 bpm], respectively for 60 and 120 s inter-set rest intervals).

Maximal blood lactate concentration displayed a moderate decrease as inter-set rest intervals increased in all studied conditions (ES: from 0.6 to 0.9). Compared to HH, NH displayed a moderate to large reduction of the blood lactate accumulation after both types of training sessions (adjusted between-group effect of 4.29 $mMol \cdot l^{-1}$ [90% CIs: 1.24, 7.33 $mMol \cdot l^{-1}$] and 3.48 $mMol \cdot l^{-1}$ [90% CIs: 0.44, 6.52 $mMol \cdot l^{-1}$], respectively for 60 and 120 s inter-set rest intervals).

Table 10 — Total volume-load during the three training sets in both groups.

		Total volume-load (Kg)							
		G1			G2			HH vs NH	
		N	HH	N vs HH ES [CI 90%] *p-value*	N	NH	N vs NH ES [CI 90%] *p-value*	Adjusted differences between hypoxia types [CI 90%]	ES [CI 90%] *p-value*
Back squat (kg)	60 s	2114.4±517.8	2123.3±468.6	**0.02 [-0.29; 0.33]** *0.904*	2142.9±240.5	2100.0±245.0	**-0.18 [-0.48; 0.13]** *0.594*	**51.75 [-135.03; 238.53]**	**0.14 [-0.75; 1.02]** *0.629*
	120 s	2111.7±522.4	2096.1±520.9	**-0.03 [-0.15; 0.08]** *0.877*	2100.0±245.0	2100.0±245.0	-	**-15.556 [-82.32; 51.21]**	**-0.04 [-0.92; 0.84]** *0.676*
	60 vs 120 s ES [CI 90%] *p-value*	**0.01 [-0.04; 0.05]** *0.976*	**0.06 [-0.21; 0.32]** *0.740*		**0.18 [-0.13; 0.48]** *0.688*	-			
Bench press (kg)	60 s	1628.3±353.1	1600.0±275.4	**-0.09 [-0.28; 0.10]** *0.608*	1529.3±307.6	1522.9±275.0	**-0.02 [-0.12; 0.07]** *0.902*	**-7.64 [-75.77; 60.49]**	**-0.02 [-0.91; 0.86]** *0.844*
	120 s	1773.3±382.3	1700.6±300.4	**-0.21 [-0.37; -0.05]** *0.053*	1537,1±288.4	1541.4±296.8	**0.02 [-0.08; 0.10]** *0.925*	**-43.05 [-110.03; 23.93]**	**-0.13 [-1.01; 0.76]** *0.277*
	60 vs 120 s ES [CI 90%] *p-value*	**-0.40 [-0.66; -0.13]** *0.009*	**-0.35 [-0.54; -0.16]** *0.027*	-	**-0.03 [-0.09; 0.03]** *0.894*	**-0.07 [-0.21; 0.08]** *0.617*	-		

G1: Group 1; G2: Group 2; N: normoxic condition; HH: hypobaric hypoxia condition; NH: normobaric hypoxia condition; 60 s /120 s: inter-set rest of the session; ES: effect size [calculated as mean difference (H-N or 120-60 s) ÷ (pooled SD) in all dependent variables; Adjusted between-group difference is the estimated marginal mean of the difference between HH and NH groups (HH group – NH group) after adjusting for N baseline differences; [CI 90%]: 90% confidence interval.

Table 11 — Mean physiological and perceptual measures recorded in both groups with different inter-set rest and conditions.

		G1			G2			HH vs NH	
		N	**HH**	**N vs HH ES [CI 90%]** *p-value*	**N**	**NH**	**N vs NH ES [CI 90%]** *p-value*	**Adjusted differences between hypoxia types [CI 90%]**	**ES [CI 90%]** *p-value*
work-HR (bpm)	60 s	150.7 ± 14.3	147.8 ± 18.5	**-0.18 [-0.46; 0.10]** *0.711*	143.9 ± 13.0	144.8 ± 12.8	**0.06 [-0.54; 0.67]** *0.908*	**3.09 [-10.69; 16.87]**	**0.20 [-0.69; 1.08]** *0.699*
	120 s	136.2 ± 17.3	136.4 ± 21.3	**0.01 [-0.16; 0.18]** *0.984*	120.2 ± 22.6	136.0 ± 13.9	**0.87 [-0.25; 1.99]** *0.097*	**0.40 [-15.15; 15.96]**	**0.02 [-0.86; 0.91]** *0.964*
60 vs 120 s	**ES [CI 90%]** *p-value*	**0.92 [0.43; 1.41]** *0.082*	**0.58 [0.24; 0.91]** *0.241*		**1.34 [0.15; 2.53]** *0.015*	**0.66 [0.14; 1.17]** *0.244*			
rest-HR (bpm)	60 s	155.9 ± 14.2	154.0 ± 17.0	**-0.12 [-0.38; 0.14]** *0.806*	139.6 ± 14.7	140.5 ± 15.5	**0.06 [-0.60; 0.72]** *0.916*	**13.56 [-0.85; 27.97]**	**0.83 [-0.09; 1.75]** *0.120*
	120 s	139.9 ± 21.0	141.2 ± 22.5	**0.06 [-0.12; 0.24]** *0.906*	110.0 ± 25.8	125.1 ± 18.1	**0.69 [-0.43; 1.81]** *0.189*	**16.12 [-1.73; 33.98]**	**0.80 [-0.12; 1.71]** *0.134*
60 vs 120 s	**ES [CI 90%]** *p-value*	**0.91 [0.47; 1.34]** *0.093*	**0.65 [0.33; 0.97]** *0.193*		**1.47 [0.27; 2.66]** *0.008*	**0.92 [0.26; 1.57]** *0.114*			
HR30 (bpm)	60 s	105.6 ± 11.9	106.5 ± 13.8	**0.07 [-0.29; 0.43]** *0.889*	96.4 ± 14.6	96.6 ± 14.8	**0.01 [-0.42; 0.44]** *0.985*	**9.95 [-2.92; 22.82]**	**0.70 [-0.21; 1.61]** *0.194*
	120 s	101.2 ± 15.5	104.5 ± 14.3	**0.22 [-0.12; 0.56]** *0.629*	88.1 ± 11.8	94.4 ± 16.6	**0.44 [-0.55; 1.44]** *0.456*	**10.03 [-4.00; 24.05]**	**0.65 [-0.26; 1.56]** *0.227*
60 vs 120 s	**ES [CI 90%]** *p-value*	**0.32 [-0.09; 0.74]** *0.494*	**0.15 [-0.04; 0.33]** *0.759*		**0.63 [-0.31; 1.56]** *0.264*	**0.14 [-0.22; 0.49]** *0.803*			

Table 11 — Continued...

		G1			G2			HH vs NH	
		N	**HH**	**N vs HH ES [CI 90%]** *p-value*	**N**	**NH**	**N vs NH ES [CI 90%]** *p-value*	**Adjusted differences between hypoxia types [CI 90%]**	**ES [CI 90%]** *p-value*
maxLac (mmol/l)	60 s	20.7 ± 4.3	19.6 ± 3.5	**-0.29 [-0.72; 0.14]** *0.531*	14.4 ± 3.6	15.3 ± 3.3	**0.25 [-0.16; 0.65]** *0.667*	**4.29 [1.24; 7.33]**	**1.25 [0.28; 2.22]** *0.027*
	120 s	16.0 ± 4.5	16.2 ± 3.7	**0.07 [-0.21; 0.34]** *0.886*	14.0 ± 3.3	12.8 ± 3.2	**-0.39 [-0.89; 0.11]** *0.526*	**3.48 [0.44; 6.52]**	**1.01 [0.07; 1.95]** *0.064*
60 vs 120 s	**ES [CI 90%]** *p-value*	**1.08 [0.57; 1.60]** *0.018*	**0.93 [0.47; 1.38]** *0.068*		**0.13 [-0.25; 0.50]** *0.843*	**0.80 [0.18; 1.38]** *0.169*			
RPE-30	60 s	8.8 ± 1.1	8.2 ± 1.1	**-0.51 [-1.16; 0.15]** *0.335*	7.6 ± 1.5	7.9 ± 1.2	**0.21 [-0.87; 1.29]** *0.675*	**0.37 [-0.68; 1.41]**	**0.32 [-0.58; 1.21]** *0.545*
	120 s	6.7 ± 1.2	6.4 ± 1.6	**-0.16 [-0.52; 0.20]** *0.739*	6.1 ± 1.1	6.0 ± 1.7	**-0.10 [-0.70; 0.50]** *0.859*	**0.44 [-1.05; 1.94]**	**0.27 [-0.62; 1.15]** *0.607*
60 vs 120 s	**ES [CI 90%]** *p-value*	**1.82 [0.81; 2.84]** *0.001*	**1.33 [0.36; 2.29]** *0.015*		**1.10 [0.32; 1.90]** *0.038*	**1.26 [0.35; 2.17]** *0.041*			
SmO_2T (%)	60 s	64.1 ± 6.3	61.2 ± 9.6	**-0.36 [-1.05; 0.33]** *0.525*	60.5 ± 11.8	42.5 ± 7.0	**-1.92[-3.05; -0.78]** *0.001*	**18.72 [11.42; 26.03]**	**2.26 [1.13; 3.39]** *0.001*
	120 s	66.3 ± 10.9	61.7 ± 17.2	**-0.33 [-0.73; 0.08]** *0.494*	52.8 ± 7.2	50.4 ± 11.6	**-0.26 [-1.22; 0.70]** *0.672*	**11.32 [-1.38; 24.01]**	**0.79 [-0.13; 1.71]** *0.139*
60 vs 120 s	**ES [CI 90%]** *p-value*	**-0.26 [-0.73; 0.21]** *0.616*	**-0.04 [-0.52; 0.45]** *0.943*		**0.81 [0.00; 1.62]** *0.132*	**-0.85 [-1.86; 0.16]** *0.154*			

60 s /120 s: inter-set rest of the session; N: normoxic condition; HH: hypobaric hypoxia condition; NH: normobaric hypoxia condition; work-HR: heart rate at work; rest-HR: heart rate at rest; HR30: heart rate during the recovery period; maxLac: maximal blood lactate; RPE: rate of perceived exertion; SmO_2T: difference between maximal and minimum value of muscle oxygenation during the three sets in total; ES: effect size [calculated as mean difference (H-N or 120-60 s) ÷ (pooled SD) in all dependent variables. Adjusted between-group difference is the estimated marginal mean of the difference between HH and NH groups (HH group – NH group) after adjusting for N baseline differences; [CI 90%]: 90% confidence interval.

As expected, ratings of perceived exertion displayed much higher values in 60 s of inter-set rest intervals with respect to 120 s in all conditions (ES: 1.43, 1.33 and 1.26 for N, HH and NH, respectively). There were no differences in the perception of the effort between both modalities of hypoxia.

» Muscle oxygenation

Similar mean SmO_2T values were detected for N and HH at both inter-set rest intervals (ES [p-value]: -0.36 [0.525] and -0.33 [0.494], respectively for 60 and 120 s). NH results displayed a moderate reduction in SmO_2T during 120 s inter-set rest intervals with respect to 60 s (ES=-0.85) (Table 2). Compared to HH, moderate to very large reductions in SmO_2T were observed in NH during both training sessions due to the reduced value in maximal SmO_2 reached in the NH group for all sets (adjusted between-group effect of 18.72% [90% CIs: 11.42, 26.03%] and 11.32% [90% CIs: -1.38, 24.01%], respectively for 60 and 120 s inter-set rest intervals) (Figure 32).

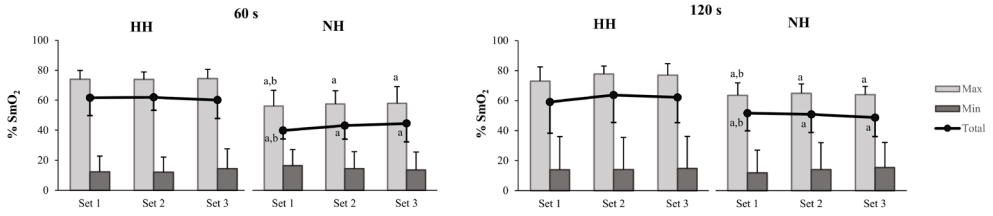


Figure 32 — Muscle re-oxygenation (max) and de-oxygenation (min) values and the difference between them (total) across the three sets for the barbell back squat. Mean and SD are represented in both hypoxic conditions (HH and NH) for 60 and 120 s inter-set rest. Significant differences ($p< 0.10$) are displayed between inter-set rest at the same environmental condition (a) and between HH and NH (b).

DISCUSSION

The aims of this study were to assess the acute effects of different types of hypoxia (terrestrial vs. simulated) during a hypertrophy-oriented resistance training session on physiological and performance markers, and to determine whether these responses are affected by alterations in the inter-set rest configuration. As expected, shorter inter-set rest periods increased perceived exertion and produced a moderate increase on cardiovascular

and metabolic responses while maintaining muscle performance capacity. Total volume-load for upper- and lower-limbs was similar in both types of hypoxia at each rest condition. For the same inter-set rest configuration, NH considerably decreased the availability of muscle oxygenation among sets and displayed a reduced maximal blood lactate concentration and mean rest-HR compared to HH. These results corroborate previous research [260] and highlight differences between types of acute hypoxic exposure on the physiological response to R_T exercise 11. There were no changes in the muscle work capacity among environmental conditions during the R_T session, although the change in the cardio-ventilatory pattern induced by the acute ascent in altitude seems to favor a more immediate recovery in HH compared to NH. Shorter inter-set rest periods produce a more stressful stimulus that, either combined or not combined with hypoxia, affect the acute response to R_T session and conceivably could maximize hypertrophic adaptations in longer periods of training.

Mechanical and metabolic stress are purported influential factors in training-induced development of muscle mass [2,64]. Inter-set rest configuration, in combination with volume and intensity, can influence the effectiveness of an acute response or chronic adaptation to a R_T program [387]. Moderate rest intervals (60-90 s) have been proposed as a viable option for maintaining a balance between mechanical and metabolic factors for gains in strength [242] and muscle size [30]. The present research compares the potential effect of a moderate rest interval (60 s) to a longer rest interval (120 s) during a traditional non-failure R_T program, that preserved mechanical stress between conditions. This outcome was verified by the fact that the total volume-load accumulated during the R_T sessions was quite similar in all environmental conditions and remained almost unaffected by the inter-set recovery periods. The trivial to moderate differences in the total volume-load of the main compared exercises (back squat and bench press) imply a lack of difference in the magnitude of mechanical stress between types of inter-set rest sessions, showing a mean difference ranged from 0.83 to 1.46 kg x set and from 0 to 0.28 repetitions x set in all conditions. Considering that the

level of recruitment seemingly cannot provide a mechanistic explanation for the physiological differences between the inter-set rest periods, other factors, such as the observed metabolic effect linked to shorter rest intervals, may be at least partially responsible for these differences [356]. Indeed, during the shorter rest intervals, perceptual, metabolic and cardiovascular responses displayed small to large increases across all conditions. As suggested in some studies, it thus is feasible that under relatively equal mechanical load, 60 s-rest intervals provide a more stressful physiological stimulus [116] that potentially could maximize the potential hypertrophic response to R_T under hypoxic conditions. Longitudinal research is needed to test the veracity of this hypothesis.

In contrast to the similarity in the performance between HH and NH in response to both R_T sessions, we observed substantial physiological effects on blood lactate accumulation, rest-HR and SmO_2T. Current evidence challenges the traditional assumption that the same inspired partial pressure of O_2 produced artificially or by a fall in barometric pressure produces similar physiological responses 11,388. Differences detected between HH and NH suggest an independent barometric pressure effect to the equated partial oxygen pressure, although as noted subsequently, the available acclimatization time to each type of hypoxia condition before exercise could also affect the physiological response.

Throughout the initial hours of exposure to moderate hypoxia there is an increase in ventilation 388,389, submaximal HR and cardiac output 369. Changes in ventilation induce hypocapnia and develop an alkalotic environment favoring the activation of the glycolytic pathway during exercise. Indeed, the reduction in circulating bicarbonate after a R_T session under hypoxic conditions [71] is interpreted as a higher buffering capacity [9,390]. The buffering response may be even more pronounced in HH than in NH due to the differences in the acute hypoxic ventilatory response 388, which may at least partially help to explain the differences observed in maximal blood lactate between both hypoxic environmental conditions. Ventilatory frequency is known to be greater in HH while the CO_2 end-tidal partial pressure is initially lower than in NH [389]. Preliminary

non-published results from our group are in accordance with this finding, showing a 4.98% higher reduction in blood bicarbonate concentration in moderate HH compared to the equivalent NH after a similar R_T session using 60 s of inter-set rest recovery (ES: 0.46; CI [-0.44, 1.36]). Note that the upper limit of the compatibility interval displays a large positive value.

Somewhat counterintuitive, but consistent with some previous research [71,295,377], our results showed a similar maximal blood lactate in N and both types of hypoxia. Blood lactate concentration conceivably should have been higher in H as result of the glycolytic pathway compensation for the reduction in O_2 availability in H [68,295], but remained similar to N due to the slower lactate release from muscle associated with an enhanced buffering response. Otherwise, at NH, maximal lactate concentration displayed a large reduction compared to HH. This decrease could be related to differences in exposure time to the hypoxic stimulus. Consistent with customary practice [68,391], acclimatization to NH only lasted 5 min before the training session. This limited time could constrain adequate activation of the cardio-ventilatory compensation mechanisms and, therefore, of the buffering response, limiting the hypoxic effect on maximal lactate accumulation. The large lower SpO_2 reached at moderate NH compared to HH just before the start of the training session is consistent with this approach (SpO_2: 94.3 and 90.7%, respectively for HH and NH, ES=-3.29, p=0.001) displaying differences in the severity of internal hypoxia achieved in each group for the same external hypoxia (FiO_2 of 16,9%) [392]. The short connection time to the hypoxic system in NH before the start of the training sessions (most frequent connection times are ranged between 5 and 10 min) could cause a greater work of breathing in the participants due to the abrupt increase in flow rates and the higher gas density, reducing the acclimatization of the ventilatory response in comparison to the HH group 388. After longer exposures (~1h), and according to the SpO_2 observed at the end of the 30 min of recovery (SpO_2: 91.7 and 94.5%, respectively for HH and NH, ES=1.35, p=0.002), desaturation is usually greater in HH [389].

To our knowledge, there currently are no data in the literature on the impact of the type of hypoxia (terrestrial vs. simulated) on muscle oxygenation. Compared to normoxia, severe NH (FiO_2 = 13%) reduces muscle oxygenation from the vastus lateralis when performing the leg press (5x10 rep; 70% 1RM; 60 s rest) [69] and from the triceps brachii after performing shoulder press and bench press (3-6 x 10 rep; ~ 75% 1RM; 60 s rest) [393]. Contrarily, similar mean relative values from the vastus lateralis oxygenation between moderate NH (FiO_2 = 15-16%) and N have been observed in other studies [295,296] after 3-5 sets x 10 repetitions (60-70% 1RM; 60 to 180 s rest) of lower-limb exercises (leg press, back squat or deadlift). These discrepancies among studies could be due to differences in the muscle assessed, type and/or severity of hypoxia when the training session is performed at simulated hypoxia. In our results, the minimum, maximum and total SmO_2 changes from the vastus lateralis were not affected by the inter-set rest duration at any environmental condition.

Compared to HH and N, moderate to very large reductions in the muscular maximal reoxygenation response during the back squat exercise were observed in NH. Surprisingly, the muscle reoxygenation capacity during the HH sets was similar to N. The accentuated increase in cardiac output and buffering capacity described in acute terrestrial hypoxia, compared to simulated hypoxia, is likely to improve glycolytic ATP production and promote muscle perfusion during recovery [368,377,388]. Moreover, the oxygen release in active muscles is favored by a rightward shift of the oxyhemoglobin curve (Bohr Effect) during exercise in H [370], which can also enhance the reoxygenation of muscle tissue at HH due to the large reduction in pH after exercise [388]. Research suggests 15-16% of FiO_2 as the minimum threshold for inducing changes in the muscle oxygenation [296,393]. Our results in NH do not support this hypothesis, although future studies are necessary to clarify the influence of the severity, type and time of exposure to hypoxia on muscle oxygenation in a similar R_T session configuration

Study 5. Effects of resistance training in hypobaric vs. normobaric hypoxia on circulating ions and hormones.

RESULTS

» Control Variables

Table 12 shows the control variables (SpO_2, lactate, and RPE) that were measured after training sessions. % SpO_2 values were significantly lower in HH than in NH, but without significant within-group differences between one- and two-minute inter-set rest. Lactate values (mmol/L) after training were significantly higher in all conditions when comparing HH with NH. There were no significant differences between-groups (HH vs. NH) in RPE, although significantly higher values were observed when a one-minute inter-set rest was used.

» Hormones and Circulating Ions

Table 13 shows the circulating ions and hormones at baseline and after training under HH and NH condition. Cortisol increased after training sessions with one-minute rest, both in HH ($p < 0.015$, ES: 0.428) and in NH ($p < 0.047$, ES: 0.495), with elevated concentrations even 30 min post-exercise. This catabolic response was accompanied by a significant increase in GH under HH ($p < 0.007$, ES: 0.568) and NH ($p < 0.003$, ES: 0.734) as well

Table 12 — Control variables after training sessions.

Groups	Inter-Set Rest (min)	SpO_2 (%)	Lactate (mmol/L)	RPE
Hypobaric hypoxia	1	92.44 ± 2.0	19.55 ± 3.5	8.22 ± 1.09
(HH) (*n* = 9)	2	92.77 ± 1.5	15.98 ± 3.89	6.44 ± 1.58 +
Total		92.61 ± 1.7 *	17.7 ± 4.0 *	7.33 ± 1.6
Normobaric hypoxia	1	95.01 ± 1.9	15.30 ± 3.3	7.8 ± 1.21
(NH) (*n* = 7)	2	93.5 ± 2.1	12.47 ± 3.2	6.0 ± 1.73 +
Total		94.28 ± 2.0 *	13.8 ± 3.4 *	6.92 ± 1.7
p-value		0.020 *	0.007 *	0.500

* $p < 0.05$. Significant differences between-groups HH vs. NH. + $p < 0.05$. Significant differences whitin-group (1 min vs. 2 min).

Table 13 — Ca^{2+}, HCO_3^-, Pi and hormones after resistance training session in hypobaric and normobaric

			HYPOBARIC HYPOXIA			*Within-Group Factor*		NORMOBARIC HYPOXIA			*Within-Group Factor*		*Between-Group Factors*		
	Rest (min)	**Baseline**	**At 5 min**	**At 10 min**	**At 30 min**	***p***	**ES**	**At 5 min**	**At 10 min**	**At 30 min**	***p***	**ES**	**Env (*HH* vs. *NH*)**	**Rest (*1 m* vs. *2 m*)**	**Env. x Rest**
Ca^{2+} (mmol/L)	1	2.37 ± 0.07	2.59 ± 0.17 *aa*	2.56 ± 0.13 *aa*	2.51 ± 0.09 *aa*	*0.004* **	0.599	2.57 ± 0.10 *a*	2.48 ± 0.13	2.44 ± 0.13	*0.040* *	0.548	***0.038* ***	0.197	0.497
	2	2.37 ± 0.07	2.64 ± 0.15 *aa*	2.59 ± 0.11 *aa*	2.65 ± 0.14 *aa*	*0.001* **	0.751	2.44 ± 0.09	2.44 ± 0.11	2.47 ± 0.09	*0.333*	0.278			
HCO_3^- (mmol/L)	1	22.70 ± 1.17	8.70 ± 2.28 *aacdd*	9.53 ± 2.75 *aabbdd*	14.70 ± 3.44 *aabbcc*	*0.001* **	0.971	10.70 ± 1.04 *aacdd*	12.05 ± 2.02 *aabdd*	15.86 ± 1.42 *aabbcc*	*0.001* **	0.987	***0.034* ***	***0.003* ****	0.733
	2	22.70 ± 1.17	11.99 ± 2.33 *aacdd*	12.46 ± 2.10 *aabdd*	17.80 ± 1.86 *aabbcc*	*0.001* **	0.965	12.67 ± 1.76 *aaccdd*	13.72 ± 2.40 *aabbdd*	16.94 ± 1.52 *aabbcc*	*0.001* **	0.983			
Pi (mmol/L)	1	2.34 ± 0.44	3.44 ± 0.92 *accdd*	3.05 ± 1.00 *bbdd*	1.73 ± 0.88 *bbcc*	*0.001* **	0.818	2.61 ± 0.77 *acdd*	2.11 ± 0.67 *bdd*	1.28 ± 0.41 *aabbcc*	*0.001* **	0.974	***0.001* ****	***0.001* ****	0.211
	2	2.34 ± 0.44	2.49 ± 0.86 *ccdd*	2.17 ± 0.81 *bbdd*	1.38 ± 0.80 *abbcc*	*0.001* **	0.688	1.17 ± 0.10 *a*	1.20 ± 0.12 *aa*	1.20 ± 0.10 *aa*	*0.001* **	0.719			
C (nmol/L)	1	431 ± 117	565 ± 76 *a*	596 ± 95 *a*	616 ± 126 *a*	*0.015**	0.428	664 ± 280 *a*	624 ± 325	620 ± 295	*0.047* *	0.495	0.083	***0.006* ****	0.899
	2	431 ± 117	416 ± 95	428 ± 116 *d*	385 ± 137 *c*	*0.056*	0.407	494 ± 174 *d*	477 ± 167 *d*	401 ± 134 *bc*	*0.370*	0.259			
GH (pg/mL)	1	398 ± 597	14542 ± 13420 *a*	14950 ± 13174 *aa*	15198 ± 13360 *aa*	*0.007* **	0.568	15559 ± 7026 *aa*	15091 ± 8464 *aa*	13971 ± 12473 *a*	*0.003* **	0.734	0.441	***0.049* ***	0.997
	2	398 ± 597	9833 ± 10883 *a*	8798 ± 9908	5105 ± 7656	*0.028* *	0.466	10248 ± 7572 *ad*	8716 ± 7404	4733 ± 4141 *b*	*0.012* *	0.650			

Between-group factors: Hypoxic environmental condition (Env), Inter-set rest (Rest) and Interaction effect: (Env x Rest). Within-group factor: a: Significant differences compared to baseline, b: Significant differences compared to 5 m; c: Significant differences compared to 10 m; d: Significant differences compared to 30 m. With double symbol of significance: $p < 0.01$; With a single symbol of significance: $p < 0.05$.

Inter-set rest factor had a main effect on C ($p < 0.006$) and GH ($p < 0.049$). No significant main effect was observed for hypoxic environmental condition factor.

The Ca^{2+} level increased significantly in all conditions at every time point after training compared to baseline. A significant main effect was observed for hypoxic environmental condition factor ($p < 0.038$).

The HCO_3^- level decreased after five minutes post-exercise, and the levels remained significantly lowered up to 30 minutes post-exercise in all conditions with a moderate-large effect (>0.90). Concentrations in NH were significantly higher than under HH ($p < 0.034$), and the values after two-minute rest were higher than after one-minute rest ($p < 0.003$).

Pi increased at five minutes post-exercise after training sessions with one-minute rest, both in HH ($p < 0.001$, ES: 0.818) and in NH ($p < 0.001$, ES: 0.974). Then, the values began to decrease progressively and significantly until reaching the baseline at 30 min post-exercise. Both inter-set rest and hypoxic environmental condition factors had a main effect on Pi; values in HH were higher than in NH ($p < 0.001$) and with one-minute than with two-minute rest ($p < 0.001$) as well.

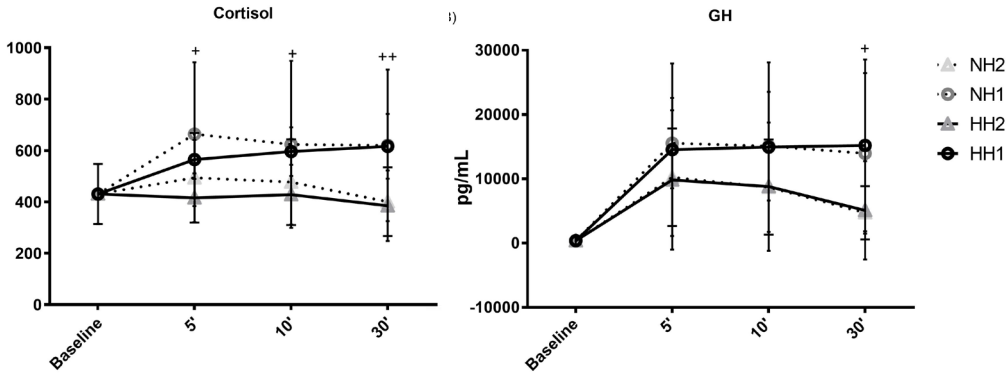


Figure 33. Hormones after resistance training sessions. Hypobaric hypoxia (HH) vs. Normobaric hypoxia (NH) with different inter-set rest (1 vs. 2 min). NH2 and NH1: Normobaric Hypoxia with 2-min inter-set rest and 1-min rest. HH2 and HH1: Hypobaric Hypoxia with 2-min inter-set rest and 1-min rest. + $p < 0.05$, ++ $p < 0.01$ comparing 1-min inter-set rest vs. 2 min rest over time. No significant differences were observed between HH vs. NH over time.

Figure 33 (hormones) and Figure 34 (circulating ions) specifically show the significant differences that exist over time in both factors (inter-set rest and hypoxic environmental condition). Regarding the inter-set rest factor, the one-minute rest compared to the two-minute rest showed significantly higher values of C (at 5, 10, and 30 min), GH (at 30 min), and of Pi (at 5 and 10 min), and significantly lower of HCO_3– (at 5, 10, and 30 min). Regarding the hypoxic environmental condition factor, the HH condition showed significantly higher values of Ca^{2+} (at 5, 10, and 30 min) and Pi (at 5 and 10 min), and significantly lower values of HCO_3– (at 10 min).

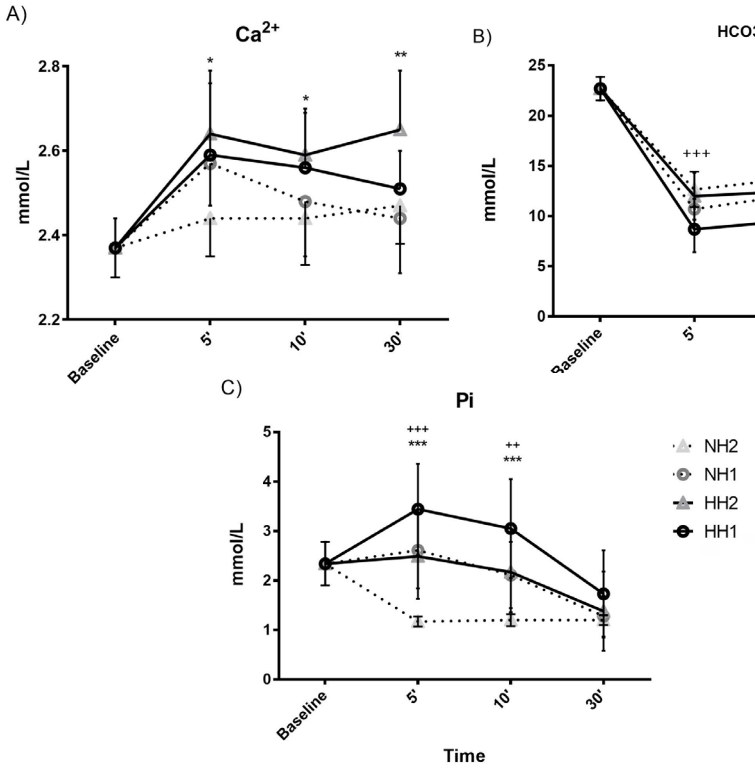


Figure 34 — Ca^{2+}, HCO_3^-, and Pi after resistance training sessions. Hypobaric hypoxia (HH) vs. Normobaric hypoxia (NH) with different inter-set rest (1 vs. 2 min). NH2 and NH1: Normobaric Hypoxia with 2-min inter-set rest and 1-min rest. HH2 and HH1: Hypobaric Hypoxia with 2-min inter-set rest and 1-min rest. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ comparing 1-min inter-set rest vs. 2 min rest over time. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ comparing HH vs. NH over time.

DISCUSSION

HH at terrestrial altitude seems to alter circulating ions related to the muscle contraction more than simulated NH, especially when the inter-set rest intervals are shorter. These findings show that in the design and periodization of high-intensity resistance training in hypoxic environments, it is necessary to pay special attention to the inter-set rest intervals.

High-intensity resistance training causes a significant acute response from serum anabolic and catabolic hormones 34,394. The results of our study are consistent with these conclusions since an increase in GH levels was observed after training, especially significant when the one-minute inter-set rest was used. The accumulation of lactate and hydrogen ions caused by resistance training stimulates anabolic hormone secretion [244]. Likewise, a significant increase in C was observed, although only after training with one-minute inter-set rest. Previous studies have concluded that the use of short inter-set rest caused an increase in metabolic stress 395. Rahimi et al. [396] concluded that serum C at 30 minutes post-exercise was significantly higher after one-minute inter-set rest than after two-minute rest. In the present research, the use of a hypoxic environment (HH or NH) did not produce any added effect on increasing GH and C levels. Woods et al. [397] stated that performing exercise in HH compared with NN produced a similar pattern of response in C. Similarly, previous studies also did not observe a significant effect of moderate hypoxia on GH and C, either in terrestrial altitude with high-intensity loads [377] or in a simulated altitude with low-intensity loads [355]. The physiological mechanisms behind the hormonal response to hypoxia is unclear and remain speculative. The hormonal changes that occur during acute exercise in hypoxia do not seem to be determined by alterations in the hypothalamic-pituitary axis, but rather by factors related to the workload of the training and the increase in lactate during exercise [398].

Circulating Ca^{2+} levels also increased after resistance training, specifically at five minutes post-exercise, without any differences between training with rest periods of one or two minutes. Resistance training is characterized by repeated skeletal muscle contractions that cause the Ca^{2+} release

ions from the sarcoplasmic reticulum into the cytoplasm. This elevation of Ca^{2+} is a consequence of the increase in the concentration of Pi that contributes to its exit from the sarcoplasmic reticulum and reduces the sensitivity of the myofilaments to Ca^{2+} during a first phase of acute fatigue 399,400. Additionally, it has been shown that intermittent exposure to hypobaric hypoxia can produce a reduction in Ca^{2+} related to the maintenance of calcium homeostasis via activation of protein kinase C isoforms [401]. However, in the present study no differences were observed in Ca^{2+} concentrations between HH and NH. In this sense, factors such as the type, duration, and intensity of exercise play an important role in the disturbance of calcium levels [402], and may be more decisive than the hypoxic environmental factor.

Contrary to what was observed for calcium levels, HCO_3^- levels decreased after training in any hypoxic environment and with both short and long inter-set rest intervals, with a tendency to return to baseline levels after 30 minutes. During intense muscular exercise, there is a significant increase in H^+ ions. To cope with this acidosis, the cells and fluids of the body have the bicarbonate buffer system, which minimizes the anaerobic production of H^+ [403]. Therefore, the decrease in HCO_3– levels indicates a muscle buffering response, especially when the inter-set rest interval is shorter. Additionally, the results also showed that training in HH caused higher decreases in HCO_3^- than training in NH. Compared to NH, HH leads to a greater hypoxemia, blood alkalosis, and a lower SpO_2 [389]. Likewise, plasma pH appears to be higher in HH than NH [304]. These physiological differences, combined with the exercise-induced metabolic acidosis, could cause changes in acid-base homeostasis and increase the muscle buffering response [404].

Serum Pi increased five minutes after performing both training sessions, in HH and NH, although only after training sessions with one-minute inter-set rest. During exercise, the muscles need energy that is obtained from ATP and cellular phosphocreatine (PCr), whose hydrolysis increases the serum Pi concentration [405]. As in our research, Luhker et al. [404] found elevated Pi levels compared to baseline up to six minutes after exercise in NH

conditions (FiO_2: 0.12). The resynthesis of ATP and the decrease in Pi concentrations appeared to be much faster after training with the longest rest periods (2 min), where at five minutes post-exercise, the levels were even below baseline. These results suggest that the one-minute inter-set rest interval was not sufficient to replenish ATP and PCr, as has been concluded in previous studies [387,406]. Regardless of the inter-set rest interval used, the Pi concentration after training in HH was higher compared to NH training, especially at five minutes post-exercise, but that difference was even maintained until 10 minutes post-exercise. Several studies have observed a greater decrease in minute ventilation, alveolar ventilation and oxygen saturation [304,389] as well as greater oxidative systemic stress 11,407 with short-term exposure to HH compared to NH. These differences could be the consequence of an increased dead space ventilation, altered fluid permeability or changes in chemo-sensitivity, probably related to the barometric pressure reduction [304]. All these factors could have increased the demands of the resistance training under HH, causing greater fatigue and greater release of Pi in the muscle fiber

Study 6. Influence of the terrestrial vs. simulated hypoxia on biomolecular anabolic signalling response to resistance exercise: A pilot study.

RESULTS

Irisin (A) and myostatin (B) changes from pre-exercise to 5 and 30 min during the recovery period are shown in Figure 35 and Table 14. The results only displayed an environmental effect in the irisin response ($p = 0.031$). R_T produced an immediate moderate reduction in irisin under HH conditions (ES = −1.02 [CI: −1.94; −0.11]) and a small non-significant increment in NH (ES = 0.56 [CI: −0.30; 1.42]). All conditions revealed small to large reductions in circulating irisin throughout the recovery, reaching the lowest values at minute 30. Circulating irisin displayed lower values in H, reaching an almost significant large decrement in HH with respect to NH at 5 minutes of the recovery period (ES = −1.10 [CI: −2.04; −0.15]; $p = 0.048$). A progressive moderate non-significant decrease below pre-exercise myostatin levels was found in both hypoxic conditions after

R_T (Figure 35B). Trivial differences in myostatin between NH and HH were observed throughout the recovery period (ES < 0.28 [CI: −0.69; 1.18]; p > 0.209). The high variability in the magnitude of the myostatin response made it difficult to identify changes in this variable. No time effect or interaction was observed in either myokine.

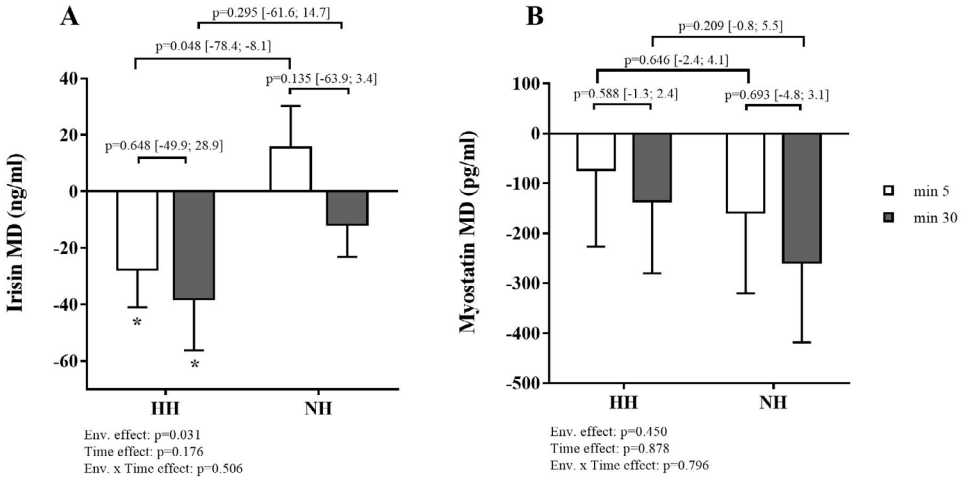


Figure 35 — Mean difference of irisin (A) and myostatin (B) 5 and 30 min into the recovery period after exercise in hypobaric hypoxia (HH) and normobaric hypoxia (NH). The p values are displayed as differences between conditions and recovery periods. * Differences to pre-exercise values (p < 0.10). Bars represent mean difference (MD) ± SEM.

The mean difference between 30 min post-exercise and pre-exercise levels of circulating miR-378 (Figure 36A), miR-206 (Figure 36B) and miR-29c (Figure 36C) are shown in Figure 36. R_T revealed moderate to large significant increments in miR-378 and miR-29c in all environmental conditions (ES between 0.66 and 2.30; p < 0.10). Compared to N, standardized mean differences indicated a rise in miR-378 for both hypoxias (ES from 0.19 to 1.55 [CI: −0.87; 2.46]), displaying only slight changes between hypoxia types. NH showed a large increase in miR-29c with respect to N (ES =1.03 [CI: 0.11; 1.94]) and HH (ES = −1.36 [CI: −2.34; −0.38]). On the contrary, miR-206 decreased slightly in N and HH after exercise to below the pre-exercise values (ES < −0.82) while displaying a clear moderate increment in NH (ES = 0.73 [CI: −0.29; 1.76] and ES = −0.96 [CI: −1.89; −0.03] compared to N and HH) (Figure 36; Table 14).

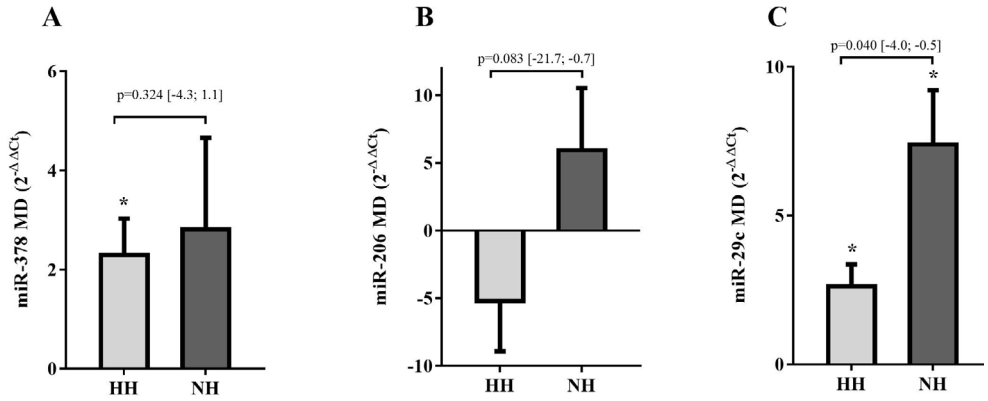


Figure 36 — Mean differences (30 min post-exercise – pre-exercise value) of (A) miR-378, (B) miR-206 and (C) miR-29c in hypobaric hypoxia (HH) and normobaric hypoxia (NH). The p values are displayed as differences between conditions. * Differences to pre-exercise ($p < 0.10$). Bars represent mean difference (MD) ± SEM.

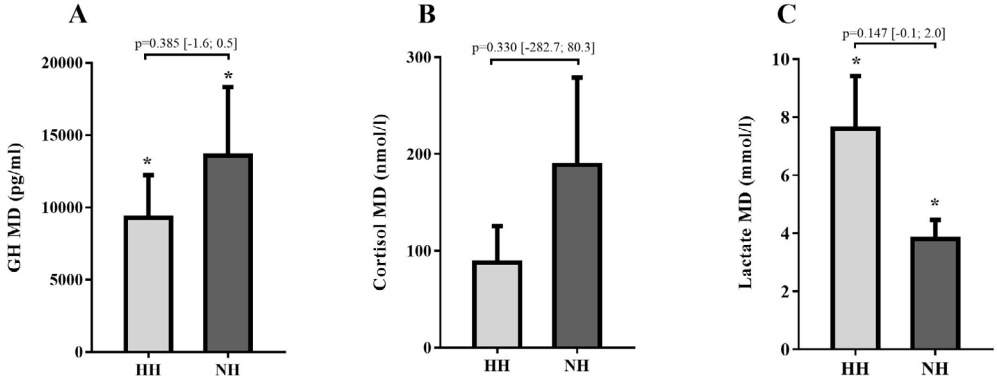


Figure 37 — Mean differences in growth hormone (GH) (A), cortisol (B) and maximal blood lactate concentration (C) 30 min into the recovery period in hypobaric hypoxia (HH) and normobaric hypoxia (NH). The p values are displayed as differences between conditions. * Differences to pre-exercise values ($p < 0.10$). Bars represent mean difference (MD) ± SEM.

Table 14 — Adjusted standardized mean differences of the studied variables between conditions.

		G1			G2			
		ΔN	**ΔHH**	ΔHH vs. N **Adj. ES** [CIs]	**ΔN**	**ΔNH**	ΔNH vs. N **Adj. ES** [CIs]	ΔHH vs. NH **Adj. ES** [CIs]
Irisin	min 5	-3.4±15.1	-27.7±13.4	-24.27 **-0.57** [-1.31; 0.17]	41.1±15.1	15.6±14.7	-25.56 **-0.49** [-1.26; 0.29]	-43.24 **-1.10** [-2.04; -0.15]
	min 30	-43.9±7.3	-38.1±18.1	5.71 **0.15** [-0.74; 1.05]	28.2±18.9	-11.9±11.3	-40.10 **-1.00 [-1-92; -0.08]**	-26.29 **-0.63** [-1.53; 0.28]
min 5 vs. min 30 **Adj. ES** [CIs]		-40.46 **-1.21** [-2.01; -0.41]	-10.48 **-0.22** [-0.66; 0.22]		-12.90 **-0.22** [-0.45; 0.01]	**-27.44** **-0.80** [-1.28; -0.31]		
Myostatin	min 5	254.0±229.1	-73.4±153.6	-327.42 **-0.57** [-0.95; -0.19]	-60.3±229.1	-158.3±161.5	-98.02 **-0.29** [-0.75; -0.18]	84.92 **0.19** [-0.69; 1.08]
	min 30	116.5±154.4	-135.6±144.2	-252.06 **-0.56** [-0.92; -0.20]	-91.1±92.5	-258.8±159.5	-167.76 **-0.50** [-0.90; -0.10]	123.24 **0.28** [-0.60; 1.18]
min 5 vs. min 30 **Adj. ES** [CIs]		-137.56 **-0.24** [-0.50; 0.02]	-62.20 **-0.14** [-0.30;0.02]		-30.79 **-0.12** [-0.48; 0.24]	**-100.52** **-0.24** [-0.42; -0.06]		
miR-378	min 30	-0.3±0.4	2.3±0.7	2.58 **1.55** [0.65; 2.46]	1.7±2.9	2.8±1.9	1.16 **0.19** [-0.87; 1.24]	-0.52 **-0.15** [-1.03; 0.74]
miR-206	min 30	-3.0±4.3	-5.2±3.7	-2.23 **-0.19** [-0.42; 0.05]	-0.8±2.3	5.9±4.6	**6.71** **0.73** [-0.29; 1.76]	-11.16 **-0.96** [-1.89; -0.03]
miR-29c	min 30	3.9±2.2	2.6±0.7	-1.32 **-0.30** [-0.96; 0.36]	2.8±1.6	7.4±1.8	4.61 **1.03** [0.11; 1.94]	-4.78 **-1.36** [-2.34; -0.38]
GH	min 30	16349.4±5034.0	9267.3±2958.1	-7082.11 **-0.59** [-1.04; -015]	11351.6±4092.3	13573.4±4746.6	2221.86 **0.19** [-0.61; 0.99]	-4306.10 **-0.40** [-1.29; 0.49]
C	min 30	134.7±36.7	87.8±37.7	-46.90 **-0.42** [-1.25; 0.41]	83.8±59.3	188.7±90.2	104.96 **0.53** [-0.50; 1.57]	-100.93 **-0.57** [-1.48; 0.33]
Lac	min 30	9.7±1.4	7.6±1.8	-2.06 **-0.43** [-087; 0.01]	5.0±1.2	3.8±0.7	-1,19 **-0.48** [-1.13; 0.17]	3.80 **1.06** [0.11; 1.99]

Data presented as Mean ± SEM (being mean = value-pre-exercise); min 5: 5 minutes of recovery; min 30: 30 minutes of recovery; N: normoxia; HH: hypobaric hypoxia; NH: normobaric hypoxia; GH: growth hormone 30 minutes of recovery; C: cortisol 30 minutes of recovery; Lac: blood lactate 30 minutes of recovery; G1: group 1; G2: group 2; Adj ES[CIs]: adjusted effect size [confidence intervals]. The adjusted Effect size was calculated as the adjusted mean change (HH-NH) or (H-N) divided by the pooled standard deviation. Values were adjusted by the corresponding pre-exercise value.

Hormonal changes induced by the different study conditions are presented in Figure 37 and Table 14. Circulating GH levels and blood lactate 30 min post-exercise increased in all conditions ($p < 0.001$) (Figure 37A). HH condition displayed a similar post-exercise GH release compared to NH (ES = −0.40 [CI: −1.29; 0.49]; $p = 0.385$). In NH, circulating blood lactate at minute 30 resulted in a large concentration increment compared to HH (ES = 1.06 [CI: 0.11; 1.99]; $p = 0.147$). Cortisol results are also shown in Figure 37B. R_T displayed moderate non-significant increments in C in all conditions (ES = 0.57 [CI: −1.48; 1.57]; $p > 0.10$). No differences in hormone response were observed between the types of hypoxia.

DISCUSSION

This study aimed to investigate the influence of a traditional hypertrophy session under different types of acute moderate hypoxia (terrestrial vs. simulated) on the myokine/miRNA response. The exercise revealed a moderate to large early decrement of irisin in HH with respect to N and NH. Compared to N, both hypoxias moderately accentuated the reduction of myostatin by a similar proportion. In all conditions, the R_T also produced increments in the studied circulating hormones, miR-378 and miR-29c. Compared to HH, increments in miR-29c and miR-206 were favoured in NH. Considering all the results together, the small to large ES detected in circulating hormones, irisin and miRNAs (29c and 206) between the two types of hypoxia could indicate differences in muscle signalling pathway activation. This hypothesis should be further investigated in future research.

R_T is largely related to mechanical- and metabolic-induced activation of muscle growth mechanisms. The hypoxic environment combined with R_T was expected to induce greater homeostasis deviation according to the reduced muscle oxygen availability [64,69,292,360] which could intensify the acute response of several modulator factors associated with muscle growth processes (such as hormones, cytokines, ROS or cellular swelling). Our results only displayed small differences in GH and C between hypoxic conditions after the exercise. Although GH release seems to be mediated by blood lactate and H^+ accumulation [69,360], it

was not affected by the higher maximal blood lactate concentration reached in HH. The pre-exercise exposure period to the hypoxic environment could affect the ventilatory response[388] and, therefore, activation of the buffer response [408] which could explain the largest increment in circulating blood lactate under this condition (the NH group was exposed for the usual standard time of 5 min while the trip to the mountain in the HH group increased the pre-exercise exposure to the hypoxic environment in ~1 h).The response of several proteins/hormones mainly linked to muscle development and metabolic homeostasis is produced by exercise-induced cytokines [409]. In this regard, myostatin acts as a negative regulator of muscle growth, playing an essential role in myogenesis[180,410,411] by the inbition of the Akt/mTOR pathway and downregulation of MyoD transcription factors[412]. Literature on research on myostatin's response to exercise in hypoxia is quite scarce. In our study, joined to the great inter-individual variability, there was a significant moderate reduction in myostatin in hypoxic conditions throughout the recovery period. This result agrees with other studies in which myostatin fell below the pre-exercise value from 3 to 24 h after a similar single[409] or longer R_T periods in N conditions[413,414]. Conversely, the effect of hypoxia on the myostatin dynamic should be expected to maintain it at a reduced level during extended R_T programmes, which could support the potential and expected interaction of FiO_2 on the relationship between myostatin and activation of the Akt/PKB-mTOR signalling cascade although this hypothesis needs to be investigated.

Irisin mainly acts in muscle-derived energy expenditure, ensuring the energy balance during exercise and probably reflecting activation of the AMPK pathway. Thus, irisin/myostatin could reflect a balance between the AMPK/Akt-mTOR pathway transition from exercise to recovery, respectively, though their relationship is intricate and not exclusive [415]. It is generally accepted that irisin increases during the first hours after acute metabolic or R_T exercise [181,409,416]. Our results showed similar irisin values after the R_T session compared to pre-exercise in N and NH, while in HH there was a significantly faster and more progressive drop just

from the end of the exercise. Other studies also found irisin values similar to pre-exercise 30 min after an isolated or full-body R_T exercise[416,417], with a predominance to increase throughout the subsequent hours[416]. Although HH is commonly presented as a metabolically more stressful context than NH [418], the better acute cardio-ventilation adaptation described in HH seems to facilitate immediate recovery from high-intensity R_T exercise [377,408], which could reduce the muscle impact of the exercise and therefore AMPK phosphorylation [415]. The large individual variability, the influence of the type of exercise [417] and the fitness level [181] in addition to the low response of irisin after R_T exercise described in the literature [409], also could modulate its dynamics after acute exercise. Nevertheless, studies conducted under hypoxic conditions on irisin response are scarce. In our research, the high fitness level of participants compared to the low ones or the lack of information in this regard in others [409,416], could also partially explain discrepancies among both types of hypoxia. This early drop in circulating irisin in HH matches with myostatin behaviour and reflects the expected balance between the AMPK/Akt-mTOR pathway transition from exercise to recovery, respectively. However, the difference between HH and NH in the dynamics of irisin/myostatin is controversial and further research on the physiological consequences of their downregulation in myogenesis after acute and long training programmes in hypoxia is needed.

Currently, several miRNAs are recognized as regulators of signalling pathways such as the IGF1/PI3K/Akt/mTOR axis, relevant to exercise adaptation. miR-378, miR-206 and miR-29c are non-coding RNAs allowing the expression of myogenic differentiation, producing activated satellite cell myogenesis and/or favouring protein synthesis[419]. Hypoxia seems to favour increments in all the examined miRNAs after the R_T exercise. In addition, the moderate to large effect of the type of hypoxia only affected circulating levels of miR-29c and miR-206 in favour of NH. Although miR-378 has been related to muscle gains after R_T in healthy recreational populations [227], it is not specifically expressed in muscle tissue, and the nature of the stimulus (exercise and hypoxia) may be a non-specific factor in the observed response [225]

in this type of participants [420]. On the contrary, miR-29c seems to be affected by the type of hypoxia, resulting in a large increment in NH with respect to HH despite being non-muscle-specific. Moreover, in murine models, miR-29c is currently considered an important repressor of the expression and function of genes involved in muscle atrophy such as MuRF1 [224]. Finally, miR-206, specifically expressed in muscle cells, has an important role in myoblast differentiation. Previous research described an acute reduction in circulating miR-206 after R_1, remaining below pre-exercise values 24 h later [212]. Our results displayed a slight tendency of a decrease in miR-206 in N and HH after exercise, which contrasts with the clear moderate increment in NH. This unexpected response clearly interferes with this research's hypothesis and with the miR-29c response in the NH group. NH seems to negatively affect the specific muscular proliferation cell process by miR-206 overexpression (reduces the expression of Pax7, Pax3 and IGF1 signalling agents) which may favour muscle cell differentiation and regeneration [419,421]. Other uncontrolled factors, such as the magnitude of the inflammatory response or muscle damage, could also interfere with these results [415]. The major impact on miR-206 and miR-29c response in NH compared to HH needs additional investigation to address complementary factors associated with the NH condition not analysed in this study.

Section IV. Chronic effect and comparison between types of hypoxia.

Study 7. Strength and muscle mass development after a resistance-training period at terrestrial and simulated intermittent hypoxia.

RESULTS

Table 15 displayed back squat and bench press 1RM and muscle thickness changes from pre-training to week 8 and week 6 of the R_T program. The results of the repeated measures ANOVA for 1 RM showed a statistically significant effect of time ($p=0.001$; $\eta^2_p>0.317$), condition ($p<0.088$; $\eta^2_p>0.150$) for both exercises, and the interaction time x condition for 1RMBP ($p=0.063$; $\eta^2_p=0.169$). Although both exercises 1RM increased after the R_T program ($p>0.001$), NH showed a large significant effect compared to N in 1RMSQ (ES=1.20). Both periods studied (weeks 1 to 6 and weeks 6 to 8) displayed similar and continuous improvements in the 1RMSQ gains (ES ranged from 0.45 to 1.01; $p<0.030$). For 1RMBP, the N group only displayed improvement in the last 2 training weeks (from the week 6 to 8; $p=0.014$) while the HH group only found gains in the first 6 weeks (from 1 to the week 6; $p=0.001$). The repeated measures ANOVA for muscle thickness showed a statistically significant effect of time ($p=0.005$; $\eta^2_p=0.041$), condition ($p<0.017$; $\eta^2_p=0.237$) and the interaction time x condition ($p=0.032$; $\eta^2_p=0.205$). Muscle growth similarly improved in N and HH groups after the R_T program (ES= -0.14; $p=1.0$). N and NH groups reached this gain in the first 6 weeks ($ES>0.46$; $p<0.024$), displaying a slight non-significant increase or reduction respectively for N (ES=0.14, $p=0.229$) and NH (ES=-0.17, $p=0.326$) groups during the last 2 weeks of the training period.

The serum GH, IGF-1 and % active mTOR changes of the adjusted to pre-training peak value in the first (S1) and the last session (S22) of the R_T program are presented in Table 16. The repeated measures ANOVA for circulating GH and IGF-1 did not display changes of interest except for the interaction time x condition in GH ($p=0.044$; $\eta^2_p=0.188$). Post-exercise GH increased in all conditions and sessions monitored ($p<0.001$). In S1, post-exercise GH release similarly favored NH and N groups ($p=0.666$; ES=0.17), reaching a large higher value in NH with respect to the HH condition

(p=0.041; ES=-1.30). Despite the IGF-1 displayed large individual variability, no significant changes were detected in serum at any moment. The results of the repeated measures ANOVA for % active mTOR showed a significant effect of time (p=0.053; η^2_p=0.119), condition (p=0.001; η^2_p=0.354) and interaction time x condition (p= 0.039; η^2_p=0.195). HH group showed a moderate to large significant increment in % active mTOR after S1 with respect to N (p=0.017; ES=1.04) and NH (p=0.002; ES=1.34).

Table 15 — Condition training effect on the absolute change in the maximal strength and muscle thickness.

	Condition	N	HH	NH	p-value [ES] Adjusted between-group differences [90% CI]		
	Weeks	Mean ± SD	Mean ± SD	Mean ± SD	ΔHH vs. N	ΔNH vs. N	ΔHH vs. NH
1RMBS (Kg)	Δ Week 8	16.0 ± 7.9	23.3 ± 9.2	27.2 ± 10.2	0.272 [0.85] 7.24 [-1.99; 16.47]	**0.023** [1.20] 11.12 [2.44; 19.81]	0.979 [-0.40] -3.89 [-12.57; 4.80]
	Δ Week 6	6.9 ± 8.4	8.9 ± 5.8	14.1 ± 12.3	1.000 [0.28] 2.04 [-7.52; 11.61]	0.247 [0.67] 7.25 [-1.75; 16.25]	0.619 [-0.52] -5.21 [-14.21; 3.79]
	Time effect: $F_{1,30}$=159.82; p=0.001; η^2_p=0.842 **Condition effect:** $F_{2,30}$=2.95; p=0.068; η^2_p =0.164 **Time x condition effect:** $F_{2,30}$=2.43; p=0.105; η^2_p =0.140						
1RMBP (Kg)	Δ Week 8	9.0 ± 5.8	10.2 ± 3.6	12.7 ± 4.7	1.000 [0.26] 1.25 [-3.51; 6.01]	0.231 [0.71] 3.67 [-0.80; 8.15]	0.708 [-0.57] -2.43 [-6.90; 2.05]
	Δ Week 6	2.5 ± 9.6	10.0 ± 3.7	7.7 ± 6.0	**0.059** [1.03] 7.49 [0.71; 14.26]	0.236 [0.67] 5.21 [-1.17; 11.58]	1.000 [0.44] 2.28 [-4.10; 8.66]
	Time effect: $F_{1,30}$=113.92; p=0.001; η^2_p =0.317 **Condition effect:** $F_{2,30}$=2.64; p=0.088; η^2_p =0.150 **Time x condition effect:** $F_{2,30}$=3.04; p=0.063; η^2_p =0.169						
Muscle thickness (VL + RF)	Δ Week 8	0.46 ± 0.31	0.41 ± 0.27	0.10 ± 0.24	1.000 [-0.14] -0.04 [-0.31; 0.30]	**0.012** [-1.30] -0.35 [-0.61; -0.1]	**0.031** [1.22] 0.31 [0.06; 0.57]
	Δ Week 6	0.37 ± 0.13	0.30 ± 0.28	0.19 ± 0.20	1.000 [-0.33] -0.07 [-0.28; 0.14]	0.168 [-1.01] -0.17 [-0.37; 0.02]	0.742 [0.44] 0.10 [-0.09; 0.30]
	Time effect: $F_{1,30}$=1.29; p=0.005; η^2_p =0.041 **Condition effect:** $F_{2,30}$=4.66; p=0.017; η^2_p =0.237 **Time x condition effect:** $F_{2,30}$=3.88; p=0.032; η^2_p =0.205						

1RMSQ 1 repetition maximum on squat; 1RMBP: 1 repetition maximum on bench press; VL: vastus lateralis; RF: rectus femoris; N: normoxia; HH: hypobaric hypoxia; NH: normobaric hypoxia; Δ Week 8: post-training value at 8 weeks – pre-training; Δ Week 6: intermediate training value at week 6 – pre-training; SD: standard deviation; p: p value for the statistical test (ANOVA); η^2_p: partial eta square; F: F test; Adjusted between-group difference is the estimated marginal mean of the difference between the environmental condition (HH vs. N; NH vs. N; HH vs. NH) at week 8 and 6 of training after adjusting for baseline differences; p-value of the adjusted between-group difference.

Table 16 — Adjusted standardized mean differences between conditions of hormones, mTOR and microRNAs.

	Condition	N	HH	NH	p-value [ES] Adjusted between-group differences [90% CI]		
	Weeks	Mean ± SD	Mean ± SD	Mean ± SD	ΔHH vs. N	ΔNH vs. N	ΔHH vs. NH
GH (pg/mL)	ΔS1	15165 ± 4460	7584 ± 1743	17113 ± 2332	0.616 [-0.71] -0.25 [-0.67; 0.18]	0.666 [0.17] 0.22 [-0.18; 0.62]	**0.041** [-1.30] -0.47 [-0.87; -0.07]
	ΔS22	11221 ± 2414	9403 ± 1681	9464 ± 1223**	1.000 [-0.28] -0.01 [-0.34; 0.33]	1.000 [-0.29] 0.03 [-0.29; 0.34]	1.000 [-0.01] -0.03 [-0.35; 0.28]
	Time effect: $F_{1,30}$=0.31; p=0.581; η^2_p =0.010 **Condition effect:** $F_{2,30}$=1.64; p=0.210; η^2_p =0.099 **Time x condition effect:** $F_{2,30}$=3.47; p=0.044; η^2_p =0.188						
IGF-1 (mg/mL)	ΔS1	22.6 ± 14.2	22.8 ± 5.7	14.0 ± 11.2	1.000 [0.01] 0.11 [-36.58; 36.80]	1.000 [-0.20] -8.64 [-43.15; 25.87]	1.000 [0.27] 8.75 [-25.76; 43.26]
	ΔS22	24.3 ± 9.3	41.6 ± 14.2	12.9 ± 11.4	0.993 [0.46] 17.31 [-21.76; 56.39]	1.000 [-0.31] -11.35 [-48.10; 25.41]	0.277 [0.67] 28.66 [-8.09; 65.41]
	Time effect: $F_{1,30}$=0.92; p=0.345; η^2_p =0.030 **Condition effect:** $F_{2,30}$=0.93; p=0.406; η^2_p =0.058 **Time x condition effect:** $F_{2,30}$=0.85; p=0.439; η^2_p =0.0.53						
% active mTOR	ΔS1	23.8 ± 9.5	147 ± 52.2	0.95 ± 4.8	**0.017** [1.04] 123.55 [31.17; 215.94]	1.000 [-0.97] -22.89 [-109.78; 64.00]	**0.002** [1.34] 146.44 [59.55; 233.33]
	ΔS22	13.9 ± 13.7	36.6 ± 27.8**	6.7 ± 6.4	1.000 [0.33] 22.73 [-32.72; 78.18]	1.000 [-0.22] -7.16 [-59.32; 45.00]	0.633 [0.50] 29.89 [-22.27; 82.05]
	Time effect: $F_{1,30}$=4.07; p=0.053; η^2_p =0.119 **Condition effect:** $F_{2,30}$=8.24; p=0.001; η^2_p =0.354 **Time x condition effect:** $F_{2,30}$=3.63; p=0.039; η^2_p =0.195						

GH: growth hormone; IGF-1: insuline-like growth factor 1; mTOR: mammalian target of rapamycin; N: normoxia; HH: hypobanc hypoxia; NH: normobaric hypoxia; ΔS1: first session of the training program – pre-training; ΔS22: last session of the training program – pre-training; SD: standard deviation; p: p value for the statistical test (ANOVA); η^2_p: partial eta square; F: F test; Adjusted between-group difference is the estimated marginal mean of the difference between the environmental condition (HH vs. N; NH vs. N; HH vs. NH) at session 1 and 22 of training after adjusting for baseline differences; p-value of the adjusted between-group difference. GH values correspond to the values of the logarithm. * Differences with respect ΔS1 in N, HH and NH (*** p < 0.001; ** p < 0.05; * p < 0.10).

Adjusted pre-exercise mean change of miR-206, miR-378 and miR-29c in S1 and S22 are displayed in Figure 38A, 38B and 38C respectively. Repeated measures ANOVA for miR-206 revealed a significant effect of the condition (p=0.026; η^2_p=0.215) and interaction time x condition (p=0.009; η^2_p=0.272). Despite not detecting a time effect, a moderate to large increase in serum miR-206 was observed from S1 to S22 in N (ES=0.76) and

HH (ES=1.65) showing no differences among groups in S22. Compared to N and HH, the NH group displayed the highest serum miR-206 value in S1 (p<0.020). The results of the non-parametric tests (Kruskal Wallis or Wilcoxon) showed a time and condition effect in miR-378 (p<0.066) and miRNA-29c (p=0.001). Compared to N and HH, the NH condition revealed moderate to large elevation of serum miR-29c in S1 and S22 (p=0.001). N and HH conditions favored a slight reduction below pre-exercise value from S1 to S22 (p<0.08).

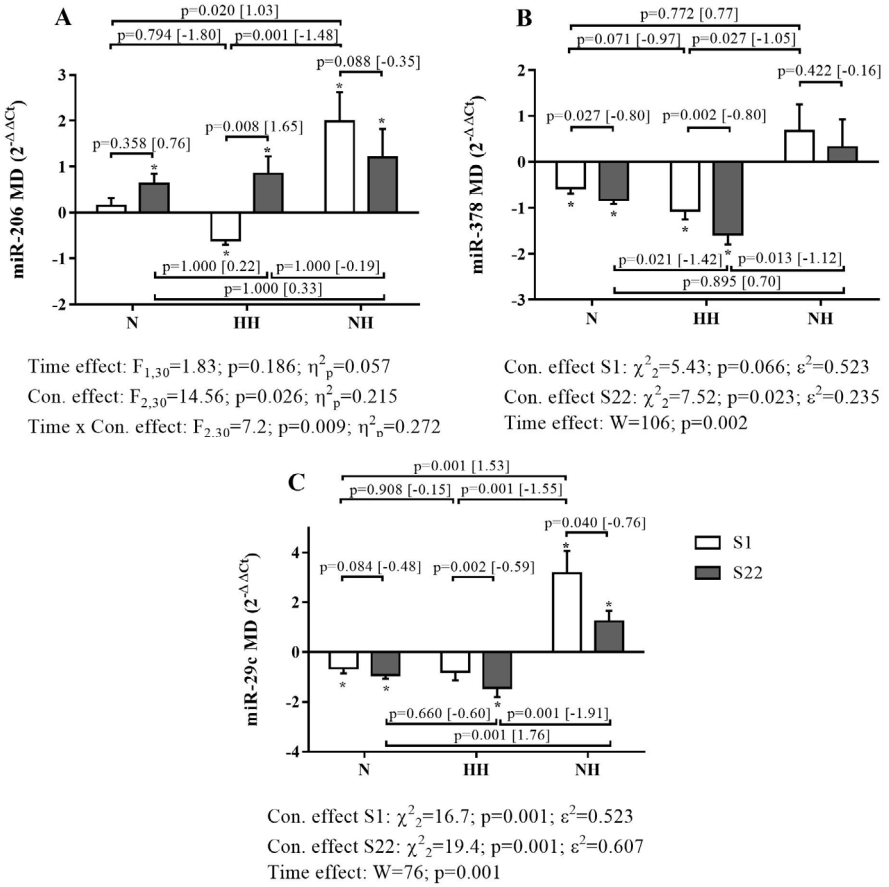


Figure 38 — Adjusted pre-exercise mean difference of miR-206 (A), miR-378 (B) and miR-29c (C) at session 1 (S1) and session 22 (S22) of the training program in normoxia (N), hypobaric hypoxia (HH) and normobaric hypoxia (NH). p values are displayed as differences between conditions or sessions. * Difference to pre-exercise values (p < 0.10). Bars represent mean difference (MD) ± SEM.

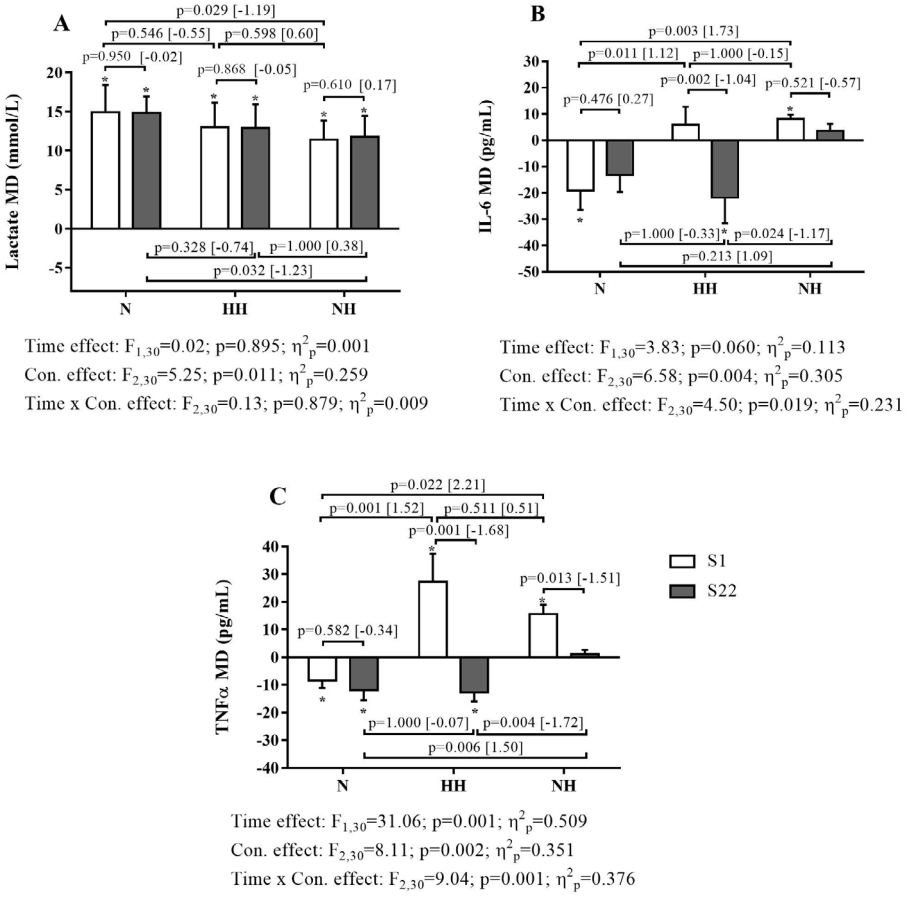


Figure 39 — Adjusted pre-exercise mean difference of lactate (A), IL-6 (B) and TNFα (C) at session 1 (S1) and session 22 (S22) of the training program in normoxia (N), hypobaric hypoxia (HH) and normobaric hypoxia (NH). The p values are displayed as differences between conditions or sessions. * Differences to pre-exercise values ($p < 0.10$). Bars represent mean difference (MD) ± SEM.

Adjusted to pre-exercise mean difference between peak values of blood lactate, IL-6 and TNFα in S1 and S22 are shown in Figures 39A, 39B and 39C respectively. Similar blood lactate increments were found after S1 and S22 in all groups ($p=0.895$; $\eta^2_p=0.001$). Pairs comparison showed significant measurements between N and NH in both testing sessions favoring N group ($p<0.032$; ES <-1.19). The results of the repeated measures ANOVA showed a significant effect of time ($p<0.06$; $\eta^2_p>0.113$), condition ($p<0.02$;

η^2_p>0.305) and interaction time x condition (p<0.019; η^2_p>0.231) for IL-6 and TNFα. Compared to N, HH and NH groups increased these two cytokines in S1 (p<0.022; ES >1.12). S22 displayed a moderate to large reduction of IL-6 and TNFα in both hypoxia to near pre-exercise values.

DISCUSSION

This study aimed to analyze the effect of an 8-week R_T period at terrestrial and simulated hypoxia on both muscle hypertrophy and maximal strength development with respect to the same training in normoxia. Main findings displayed the highest increments in % active mTOR in the HH group and the greatest inflammatory response (IL-6 and TNF-α) and circulating miRNAs linked to muscle growth (miR-206, miR-378 and miR-29c) throughout the training period in the NH group. However, main gains in muscle thickness favored N and HH groups over NH, which do not support the expected additional benefit of RTH over RTN on muscle growth. Differences between both types of hypoxia were found and place the NH condition as the most disadvantageous position for muscle growth, although it seems to favors strength development by factors not assessed in this study.

RTH is expected to enhance muscle growth and strength[292,300,353]. Contrary, the results of this study show an increase in muscle thickness of the lower limbs in N (8.03%) and HH (5.49%), while the NH group hardly found a change (1.83%) (Table 15). Available research reveals unclear muscle growth results after training periods in moderate to severe NH, regardless of the assessment method (CSA, muscle thickness, or lean mass)[9,422]. The benefit of hypoxia in muscle hypertrophy is associated with a higher metabolic stress during exercise[67,84], triggering several mechanisms related to protein synthesis and the activation, proliferation and differentiation of muscle satellite cells [415,423,424]. Moreover, the rise in H^+ augments the frequency of discharge of group III/IV muscle afferents[84,425] enhancing the type II fibers recruitment, more susceptible to hypertrophy and strength development, to protect against conduction to failure and compensate for the loss of force and velocity[63,426]. In this regard, our three study groups largely increased post-exercise blood lactate. The NH group registered

moderate to large values below the reached in the HH and N groups respectively (Figure 39), probably by the incomplete adaptation of the ventilatory system in NH before the exercise and its impact on lactate dynamics (i.e., glucose metabolism, oxyhemoglobin dissociation curve and buffer activity) widely described in other studies[420]. However, although the NH group also revealed the lowest muscle growth, this condition displayed the largest 1RMSQ improvement after the training (Table 15). Some studies also found significant improvements in maximal strength gains after 3 to 7 weeks of R_T under simulated hypoxic conditions [300,350,351], while others not[345,352,354]. Recent meta-analysis and systematic reviews have repeatedly failed to demonstrate an additional benefit of RTH compared to RTN[7–10,422]. Discrepancies among studies' training protocols are suggested as the main cause of inconsistent results. The results of this study support this benefit neither. An advantage of RTH has only been found in improving strength, regardless of muscle structure changes, due to causes probably linked to neuromuscular adaptations not analyzed in this study. Moreover, all the available literature on the topic was conducted under NH, being this research, to our knowledge, the first of these characteristics conducted under terrestrial and simulated hypoxia for comparative purposes.

The first (S1) and the last (S22) session of the R_T period show elevated blood lactate and circulating GH in all groups. Consistent with previous research, S1 in HH displayed moderate to large lower increments of GH compared to N and NH[377,420,427]. The release of H^+ into the bloodstream highly affects the GH response[116,139]. The elevated ventilatory response in hypoxia[388,389] induces hypocapnia and develops and alkalotic environment reducing the pH. The longest pre-exercise exposure time in HH could settle the hypocapnic response and increase the pre-exercise pH compared to NH[71,377], which potentially can explain the physiological reduction of GH release after S1[138]. Otherwise, circulating GH was similar between conditions at the end of the R_T program, showing a good adaptive response to exercise training (see Table 16).

Although GH primarily potentiates the release of IGF-1[141], our results

did not show changes in this hormone by the training program in any group. Post-exercise IGF-1 response shows inconsistent results in the literature displaying increases[347] or no change[353] in simulated hypoxia and similar increases between hypoxia and normoxia conditions[69]. The IGF-1 production from the liver and active muscle can be autocrine and therefore, undetectable in blood[226], almost explaining these discrepancies among studies and the lack of consistency found between GH and IGF-1. Moreover, the IGF-1 carries out signaling through multiple anabolic cascades, including IGF-1/PI3K/AKT/mTOR[93] and directly mediate synthesis of muscle proteins. The results displayed a % active mTOR largely upregulated at the beginning of the R_T period in HH condition, although this difference concerning N and NH seems not to be enough to discriminate the net muscle mass gains between groups at the end of the training period. Other mTOR signaling pathway research found a downregulation[428,429] or unaltered values 3 hours after a R_T exercise in severe simulated hypoxia[288]. We do not have references data from a shorter time window as used in this study. Nevertheless, the role and contribution of single measurements of IGF-1 and mTOR remains speculative due to the multiple targets and mechanisms involved in the signaling processes[105].

In agreement with previous studies[226,420] the results displayed a fast R_T-induced inflammatory response (IL-6 and TNFα) in both hypoxic conditions after the first training session (S1) while remained downregulated or near to pre-training in N and after the last one (S22) in all conditions. The increase in ROS production and HIF-1α expression[430], joined to accentuated anaerobic metabolism during the exercise in hypoxia[84], could limit the ability to resynthesize glucose and explain the acute TNFα overexpression found in HH and NH after S1. Moreover, the high systems stimulation to unaccustomed conditions (such as a hypoxic environment) may impair sustained elevations in the inflammatory response and reduce it when muscle adapts to training, as was detected in S22 in all conditions[431]. The maintenance of serum IL-6 above the pre-training values in NH contrasts again with the small increase in muscle thickness recorded in this group. Therefore, the time-course

exploration of the inflammatory response and the relationship between inflammatory cytokines and muscle adaptations in both hypoxia type need further exploration.

Finally, overexpression of miR-206, 378 and 29c have been associated to muscle differentiation, activation of satellite cells, myogenesis and/or protein synthesis promotion[224,419,421]. In accordance with other research[212,427], the beginning of the trained period displayed a large increment of circulating miR-206 in NH and displayed a progressive increase throughout the training period in the rest of groups until reaching similar above pre-exercise values at the end of the program. The NH condition was the only one that maintained high circulating miR-29c values throughout the training period. MiR-206 overexpression in NH could negatively affects muscular satellite cells proliferation, although it may favor muscle cell differentiation and regeneration[419,421]. Moreover, the unexpected progressive decrease of miR-378 and 29c in N and HH with respect to the exacerbated elevation in NH condition refers to an impact of the type of hypoxia to a similar R_T program. Increments in circulating miR-378 has been linked to muscle growth after a R_T routine in untrained subjects[227]. However, this rise may reflect a global body response in low physical condition population to whatever exercise rather than specifically to muscle hypertrophy, since similar responses are observed after other types of exercise[229,386]. Therefore, changes in miR-378 may not be aligned with muscle mass development in trained populations as the used in this study.

It should be noted that differences between hypoxic stimulus (i.e., HH vs. NH) can produce the greatest circulating miRNAs (miR-29c and miR-206) in NH and promote non-hypertrophy-directed changes, but instead a potentiated muscle repair-oriented process. We considered the possibility that some not assessed air quality factors linked to the NH-generating device used in this study could affect net stress supported by participants of this group in addition to the reduced FiO_2. The NH tent is a quite reduced space susceptible to accumulating big rises of carbon dioxide (CO_2), temperature and humidity inside during high-intensity exercise[432], affecting the SpO_2 for the same FiO_2[433,434]. We measured the relative

humidity (%RH) and CO_2 on different occasions before and after the training sessions during the study. Inside the NH tent, the % RH ranged from 60 to 90%, and the CO_2 from 1488 to 6374 ppm; which contrast with the <1100 ppm of CO_2 and the ~28% RH measured in the HH gym, without affecting the expected work capacity in any case. Future studies should ensure or, at least, describe the air quality to avoid the impact of uncontrolled stressors when using NH devices on the outcomes. Moreover, the individual sensibility to the hypoxia[392] and to the hypertrophy training (low or high responders)[227] type of muscle fiber predominance[220], or even the miRNA time-course of the response could entail an additional stimulus to the R_T program that should be further investigated.

5

General Discussion and Conclusions

5.1. General discussion

The aim of this thesis was to analyze the acute and chronic effect of the R_T under two types of hypoxia conditions (terrestrial vs. simulated) on muscle mass and strength performance. Accordingly, the set of studies conducted have helped to improve our understanding of the role of the type of hypoxia on serum biomarkers associated with muscle growth and strength development. Several studies were carried out to 1) analyze the acute effect of different types of hypoxia and inter-set rest configurations on serum and muscle performance markers (Section III), and 2) to analyze the chronic effect of different types of hypoxia on serum markers and muscle performance (Section IV). Other preliminary studies (Section II) and a systematic review and metanalysis on the topic (Section I) allowed to determine the current status of knowledge of RTH on muscle hypertrophy and to refine the methodological procedures employed concerning the structure of the sessions, blood extraction and measurements procedures.

The acute response (Section III) suggests that the metabolic and physiological responses of a hypertrophic R_T exercise in hypoxia are mediated by rest intervals between sets and the type of hypoxia. Shorter intaer-set rest periods are linked to an increased metabolite accumulation[2,356] that potentially could maximize the hypertrophic response to R_T under hypoxic conditions. Indeed, during the shorter rest intervals (60 s), perceptual, metabolic and cardiovascular responses displayed small to large increases across all conditions compared to longer inter-set rest intervals (120 s). Moreover, the combination of R_T with a hypoxic environment was expected to induce greater homeostasis deviation according to the reduced muscle oxygen availability[64,69,292,360] which could intensify the acute response of several modulator factors associated with muscle growth mechanisms (such as hormones, cytokines, ROS or cellular swelling). Accordingly, the results displayed an intensification in the pro-/anti-inflammatory response joined to an accentuated anaerobic metabolism during the acute R_T exercise in hypoxia[84]. The marked and early reduction in myostatin in hypoxia could reflect

the expected activation of the AKT-mTOR pathway in benefit to muscle growth. In contrast to the similarity in the performance between HH and NH in response to the same R_T session, data revealed substantial differences in blood lactate accumulation, rest-HR, SmO_2, testosterone, Ca^{2+}, Pi and miRNAs (miR-378, miR-206 and miR-29c) between groups[408,418]. Although HH seems to develop greater muscular fatigue and metabolic than NH[418], this last condition shows higher circulating values of specific markers related to muscle differentiation (i.e., miR-206)[427]. Therefore, hypoxia condition seems to favor the mechanisms linked to muscle growth more than normoxia. However, differences between both types of hypoxia could affect net muscle growth at the end of a training period. These results support the current evidence that challenges the traditional assumption that the same FiO_2 produced artificially or by a fall in barometric pressure produces the similar physiological response[11,388]. The available acclimatization time to each type of hypoxia condition before exercise could also affect the physiological response favoring HH condition[260].

The chronic response obtained after a R_T period of 8-weeks (Section IV) does not support the expected added benefit of RTH compared to RTN on muscle mass development, although it seems to favour gains in strength. The benefit of hypoxia in muscle hypertrophy is associated with higher metabolic stress during exercise[67,84], enhancing the type II fibers recruitment, more susceptible to hypertrophy and strength development[63,426]. The NH group registered moderate to large blood lactate values below the reached in HH and N groups, which could indicate a limitation in the net metabolic stress response and the associated hypertrophy mechanisms, placing the NH condition in a more disadvantageous position for muscle growth than HH. A higher inflammatory response (IL-6 and TNF-α) and circulating miRNAs (miR-206, miR-378 and miR-29c) related to muscle growth have been found in the NH group. However, miR-206 overexpression in NH could negatively affect muscular satellite cell proliferation, although it may favor muscle cell differentiation and regeneration[419,421].

It should be noted that these results apply to an intermittent moderate hypoxic strategy in resistance-trained males, thus the outcomes cannot be extrapolated to continuous periods, more severe hypoxic stimulus, different training status or populations. It should also be kept in mind that some variables related to air quality linked to the NH-generation device used could affect the net stress of the NH group and the results. Future research should elucidate the impact of R_T and the role of hypoxia on serum biomarkers associated with muscle growth and the adaptation of other non-structure factors related to muscle strength development.

The findings of this thesis should be interpreted taking these two aspects into account. On the one hand, the outcomes remain as close as possible to real-life R_T situations to which athletes are exposed daily, so they have a high ecological value. On the other hand, the windows time used for blood extractions after the training sessions is a limitation. Moreover, the complexity of the activation signaling pathways involved in myogenesis entails other limitations when understanding the mechanisms by which the expected extra increase in muscle mass does not occur under hypoxic conditions. The current work may serve as a basis for future research aiming to address the differences between types of hypoxia on R_T and estate basic rules in the correct use of different O_2 content environments on R_T in sports populations.

5.2. Conclusions

» **Study 1**

Consistent with previous reviews, the overall pooled results remained inconclusive as to the use of RTH compared to RTN for muscular adaptations, despite the inclusion of a substantial body of new research on the topic. The findings of this systematic review and meta-analysis provide insights for the prescription of resistance training at intermittent systemic hypoxia exposure to promote muscular hypertrophy and strength development. The subgroup analysis revealed 2 conditions under which the use of R_T in hypoxia may be of benefit: 1) training programs that employ loads between 60-80%1RM, inter-set rest intervals of ≤60 s and moderate hypoxia show greater increases in muscle CSA;

2) training programs that employ loads between 60-80%1RM and inter-set rest intervals ≥120s show greater increases in strength; however, the severity of hypoxia does not appear relevant to gains in 1RM .

» **Study 2.**

In conclusion, the exposure to acute moderate terrestrial hypoxia during a whole-body hypertrophy-oriented R_T session does not appear to differentially affect the metabolic stress response compared to R_T in normoxia. The acute response observed in this study therefore cannot necessarily be extrapolated to longer periods of exposure, raising the possibility that a more stressful response to the same exercise occurs after the initial acclimatization phase. Future studies should seek to identify the mechanisms involved in the hypertrophic response and clarify the effect of prolonged continuous or intermittent exposure to moderate altitude combined with longitudinal R_T programs on muscular adaptations

» **Study 3.**

In conclusion, a traditional hypertrophic R_T program performed at acute moderate hypoxia does not seem to provide a more potent hormonal or inflammatory response that could enhance the muscle growth stimulus to a greater extent than equivalent training in normoxia. However, the standardized mean difference values in the myokines studied point to an intensification of the inflammatory response in H that should be further investigated in relation to the assessment of athletes' health and the role they have on activating muscle adaptations to strength training. Although the acute muscle adaptative response to this type of R_T seems not to be mediated by changes in circulating levels of miR-378, the link observed with the GH and C could indirectly indicate the miR-378 relationship with the expression of other target genes involved in R_T-induced muscle hypertrophy. The response observed cannot necessarily be extrapolated to longer time periods and types of hypoxia (e.g., terrestrial vs. simulated).

» **Study 4.**

In conclusion, shorter session's inter-set rest intervals (60 s) provide a more potent cardiovascular and metabolic stimulus and intensify the perceptual response in all environmental conditions. For an equivalent FiO_2, the

type of hypoxia (terrestrial vs. simulated) affects the physiological response to a traditional hypertrophy-oriented R_T session. The improvement in buffering capacity and rest-HR at HH favors a better inter-set recovery compared to NH, with findings more prominent as the rest intervals shorten. In addition, it is possible that the 5 min of pre-exercise acclimatization time provided in NH constrained the activation of the physiological compensation mechanisms affecting the muscle oxygen saturation.

» Study 5.

High-intensity resistance training with one-minute inter-set rest causes greater hormonal stress (C and GH) than with two-minute inter-set rest interval, although HH does not seem to have a significant effect on these hormones. Furthermore, HH at terrestrial altitude seems to alter circulating ions related to the muscle contraction more than simulated NH, especially when the inter-set rest intervals are shorter. These results must be taken into account when planning resistance training in hypoxia, since the inter-set rest interval and the hypoxic environmental condition could be decisive to elicit different metabolic and physiological responses during the training sessions.

» Study 6.

The type of hypoxia affects the response of circulating hormones, irisin and specific miRNAs after a hypertrophic R_T exercise, which could indicate differences in muscle signalling pathway activation, with no effect on muscle mechanic capacity. From a general point of view, the response of myostatin and miR-29c in hypoxia clearly seems to stimulate expression of genes linked to myogenic processes. However, the overexpression of miR-206 under NH conditions reveals a potential influence of this type of hypoxia on muscle signalling pathways, which could be almost interpreted as a tendency to more pronounced muscle preservation because of the type of hypoxia that could limit muscle growth after longer periods. Further research is needed to clarify the impact of the type of hypoxia on biomolecular response linked to muscle growth and remodelling over extended training periods

» Study 7.

In conclusion, 8 weeks of R_T do not support the hypothesis of the

potential additional benefit of RTH on muscle growth, although seems to favor strength gains. The greater muscle growth achieved in HH over NH confirms differences between both types of hypoxia, which is also reflected in the acute and chronic response of some biomarkers, such as miR-206 and miR-29c without affecting performance. Some variables related to air quality and linked to the NH-generating device used in this study could affect the net stress of the NH group, added to the reduced FiO_2, affecting these results. Future research needs to elucidate further the impact of R_T and the role of the type of hypoxia on serum biomarkers associated with muscle growth, as well as in the adaptation of other factors not linked to the muscle structure related to the gain of muscular strength.

5.3. Conclusiones

» Estudio 1.

De acuerdo con las revisiones anteriores, y a pesar de la inclusión de nuevas investigaciones sobre el tema, los resultados no son concluyentes en relación a las adaptaciones musculares producidas por el entrenamiento de fuerza en hipoxia en comparación con el mismo tipo de entrenamiento en normoxia. Los hallazgos de esta revisión sistemática y metaanálisis permiten dar información para la prescripción del entrenamiento de fuerza en hipoxia intermitente con el objetivo de mejorar la hipertrofia muscular y el desarrollo de la fuerza. El análisis por subgrupos reveló 2 condiciones por las cuales podría mejorarse el entrenamiento de fuerza en hipoxia: 1) programas de entrenamiento con cargas entre 60-80% 1RM, intervalos de descanso entre series de ≤60 s e hipoxia moderada muestran mayores aumentos en la sección transversal del músculo; 2) los programas de entrenamiento que emplean cargas entre 60-80% 1RM e intervalos de descanso entre series ≥120 s muestran mayores aumentos en la fuerza; sin embargo, la severidad de la hipoxia no parece relevante para las ganancias en 1RM.

» Estudio 2.

La exposición aguda en HH moderada durante una sesión de entrenamiento orientada a la hipertrofia (full-body con 6 ejercicios: 3 series x 10 RM; 2 min de recuperación) no parece afectar a la respuesta de estrés metabólico de manera diferente a ese mismo entrenamiento en normoxia. Sin embargo,

la respuesta aguda observada no debe necesariamente extrapolarse a períodos más largos de exposición, en los que es posible que se intensifique la repuesta de estrés una vez pasada la fase de aclimatación inicial.

» Estudio 3.

Una sesión de entrenamiento orientada a la hipertrofia en HH moderada no parece proporcionar una respuesta hormonal o inflamatoria que indique una mejora del estímulo de crecimiento muscular con respecto a la condición de normoxia. Sin embargo, las diferencias de medias estandarizadas obtenidas para las mioquinas estudiadas sugieren que esta respuesta inflamatoria es más intensa en HH, lo que podría suponer un papel fundamental en la activación de las adaptaciones musculares al entrenamiento de fuerza. Aunque la respuesta aguda de adaptación muscular a este tipo de entrenamiento parece no estar mediada por cambios en los niveles circulantes de miR-378, la relación observada de este miRNA con la GH y el cortisol podría indicar una asociación de la expresión del miR-378 con otros genes diana implicados en la hipertrofia muscular inducida por el entrenamiento. La respuesta observada no debería extrapolarse a un periodo de entrenamiento o entre diferentes tipos de hipoxia (terrestre frente a simulada).

» Estudio 4.

Intervalos de descanso entre series más cortos (60 s vs. 120 s) intensifican la respuesta perceptiva, cardiovascular y metabólica en todas las condiciones ambientales (N, HH y HN). Para una FiO_2 equivalente, el tipo de hipoxia (terrestre vs. simulada) afecta a la respuesta fisiológica de una sesión de entrenamiento orientada a la hipertrofia. La mejora en la capacidad "buffer" y en la FC de descanso en HH parece mejorar la recuperación entre series en comparación con HN, obteniendo resultados más elevados cuando los intervalos de descanso son más cortos. Además, es posible que durante los primeros 5 min de aclimatación en HN haya una limitación en la activación de los mecanismos de compensación fisiológica que afectan a la saturación de oxígeno muscular.

» Estudio 5.

Las respuestas metabólicas y fisiológicas del entrenamiento de fuerza orientado a la hipertrofia están mediadas por el intervalo de descanso entre

series (60 s vs. 120 s) y la condición ambiental (HH vs. HN). Aunque la respuesta del cortisol y de la GH a este tipo de esfuerzo no parece afectarse por el tipo de hipoxia, la HH parece alterar en mayor medida la concentración de iones circulantes relacionados con la contracción muscular en comparación a la HN, especialmente con intervalos cortos de descanso. Estos resultados deben tenerse en cuenta al planificar el entrenamiento de fuerza en hipoxia, ya que el intervalo de descanso entre series y el tipo de hipoxia podrían ser determinantes para provocar diferentes respuestas metabólicas y fisiológicas durante las sesiones de entrenamiento pudiendo afectar al resultado en periodos de tiempo más largos.

» Estudio 6.

El tipo de hipoxia (HH vs. HN) afecta la respuesta hormonal, de irisina y miRNAs específicos de una sesión de entrenamiento de hipertrofia, lo que podría indicar diferencias en la activación de la vía de señalización muscular. Desde un punto de vista general, los niveles de miostatina y miR-29c en hipoxia parecen estimular la expresión de genes relacionados con procesos miogénicos. Sin embargo, la sobreexpresión de miR-206 en HN revela una influencia potencial sobre las vías de señalización muscular que podría interpretarse como una tendencia a una mayor preservación del músculo asociada al tipo de hipoxia, pudiendo limitar el crecimiento muscular en períodos más largos. Se necesita más investigación para aclarar el impacto del tipo de hipoxia en la respuesta biomolecular relacionada con el crecimiento y la remodelación muscular durante períodos de entrenamiento prolongados.

» Estudio 7.

Ocho semanas de entrenamiento de fuerza orientado a la hipertrofia no respaldan la hipótesis del beneficio potencial del entrenamiento de fuerza en hipoxia sobre el crecimiento muscular, aunque parece favorecer la ganancia de fuerza. El mayor crecimiento muscular alcanzado en HH con respecto a HN confirma que hay diferencias con respecto al tipo de hipoxia, lo que también se refleja en la respuesta aguda y crónica de algunos biomarcadores evaluados tales como el miR-206 y miR-29c, sin que su modulación afecte al rendimiento. Establecemos la hipótesis de que variables relacionadas con la calidad del aire y vinculadas al dispositivo generador

de HN utilizado en este estudio podrían afectar al estrés neto soportado por el grupo de HN como añadido a la reducida FiO_2. La investigación futura debe dilucidar aún más el impacto del entrenamiento de fuerza y el papel de la hipoxia en los biomarcadores séricos asociados con el crecimiento muscular, así como en la adaptación de otros factores no relacionados con la estructura vinculados con la ganancia de fuerza muscular.

6

Limitations

Limitations of these studies can be addressed:

► The duration of the post-exercise recovery period lasted only 30 min, thus limiting the ability to draw conclusions beyond this acute time frame.

► A double-blind design could not be employed in the HH group due to the intrinsic characteristic of the terrestrial altitude. To reduce the potential for confounding, participants were not informed about the expected hypoxic effect on performance.

► Our sample size was relatively low, which may have compromised the ability to detect statistical significance in some outcomes.

► Some variables related to air quality and linked to the NH-generating device used in the studies could affect the net stress of the NH group, added to the reduced FiO_2, affecting the results.

7

Future Research

Suggestions for future research directions in the field of hypoxia and R_T could comprise:

► Promote greater standardization of training protocols that better reflect applicability to participant's training status.

► Explore the effect of R_T under continuous or intermittent exposure to terrestrial hypoxia, whose physiological responses differs from breathing O_2-depleted air (normobaric hypoxia).

► Clarify the impact of the type of hypoxia on biomolecular response linked to muscle growth and remodeling over extended training periods under continuous or intermittent exposure.

► Corroborate the impact of the type of hypoxia on muscle adaptations, biomolecular and genetic responses after and during a R_T program.

► Include women in the samples to better representation of the population.

► Isolate the impact of additional stressors associated to the air quality linked to NH devices to better understand the influence of hypoxia in neuromuscular and structural adaptations to R_T.

8

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9

Appendices

Table S1. PRISMA for abstract checklist.

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes

Section and Topic	Item #	Checklist item	Reported (Yes/No)
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	N/A

Table S2. PRISMA checklist.

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Yes
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Table S1 Yes
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Yes
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Yes
Information sources	6	Specify all databases, registers, websites, organizations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Yes
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Yes Figure 18

Section and Topic	Item #	Checklist item	Location where item is reported
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Yes Table S3
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Yes Figure 18

Section and Topic	Item #	Checklist item	Location where item is reported
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Table 6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Table S3 Yes
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Table S4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Yes Table S5

Section and Topic	Item #	Checklist item	Location where item is reported
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Yes
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Yes
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Yes
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Yes
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Yes
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Yes

Section and Topic	Item #	Checklist item	Location where item is reported
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Yes
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Yes
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Yes
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Yes
Study characteristics	17	Cite each included study and present its characteristics.	Yes
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Table S4
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Yes

Section and Topic	Item #	Checklist item	Location where item is reported
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Yes
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Yes
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Yes
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Yes
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A

Section and Topic	Item #	Checklist item	Location where item is reported
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Yes
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Yes
	23b	Discuss any limitations of the evidence included in the review.	Yes
	23c	Discuss any limitations of the review processes used.	Yes
	23d	Discuss implications of the results for practice, policy, and future research.	Yes
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	N/A
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	N/A
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A

Section and Topic	Item #	Checklist item	Location where item is reported
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Yes
Competing interests	26	Declare any competing interests of review authors.	Yes
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Appendices

Table S3. Search strategy used in each database.

Database	Search strategy
Pubmed-Medline	("strength training"[All Fields] OR "resistance training"[All Fields] OR "weight training"[All Fields]) AND ("hypoxia"[MeSH Terms] OR "hypoxia"[All Fields] OR "hypoxia s"[All Fields] OR "hypoxias"[All Fields] OR ("altitude"[MeSH Terms] OR "altitude"[All Fields] OR "altitudes"[All Fields]) OR (("hypoxia"[MeSH Terms] OR "hypoxia"[All Fields] OR "hypoxic"[All Fields] OR "hypoxical"[All Fields] OR "hypoxically"[All Fields]) AND ("education"[MeSH Subheading] OR "education"[All Fields] OR "training"[All Fields] OR "education"[MeSH Terms] OR "train"[All Fields] OR "train s"[All Fields] OR "trained"[All Fields] OR "training s"[All Fields] OR "trainings"[All Fields] OR "trains"[All Fields]))))
Web of Science	(TS=((("strength training") OR ("resistance training") OR ("weight training")))) AND TS=(((hypoxia) OR (altitude) OR (hypoxic training)))
Sport Discuss	(("strength training") OR ("resistance training") OR ("weight training")) AND ((hypoxia) OR (altitude) OR (hypoxic training))
Scopus	TITLE-ABS-KEY ((("strength training") OR ("resistance training") OR ("weight training")) AND ((hypoxia) OR (altitude) OR (hypoxic AND training)))

Table S4. Risk of bias and quality assessment.

Study	Specification of eligibilit	Random sequenc	Allocation conceal	Inter-group similarit	Blinding of particip	Blinding of outco	Incomplete outco	Selective report	Total quality
Chycki (2016)	Low	Low	N/A	Low	Low	N/A	Low	High	5/6
Fashi (2020)	Low	Low	N/A	N/A	Low	N/A	Low	Low	5/5
Friedman (2003)	Low	Low	N/A	Low	Low	N/A	Low	High	5/6
Ho (2014)	Low	N/A	N/A	Low	Low	N/A	Low	Low	5/5
Honda (2020)	Low	Low	N/A	Low	N/A	N/A	Low	Low	5/5
Inness (2016)	Low	N/A	N/A	N/A	Low	N/A	Low	High	3/4
Kon (2014)	Low	Low	N/A	Low	N/A	N/A	Low	Low	5/5
Kurobe (2015)	Low	Low	N/A	Low	Low	N/A	Low	Low	6/6
Manimmanakorn	Low	Low	N/A	N/A	N/A	N/A	Low	Low	4/4
Martínez-	Low	Low	N/A	Low	Low	N/A	Low	Low	6/6
Martínez-	Low	Low	N/A	N/A	Low	N/A	Low	Low	5/5
Mayo (2018)	Low	Low	N/A	Low	Low	N/A	Low	Low	6/6
Nishimura (2010)	Low	Low	N/A	Low	High	N/A	Low	Low	5/6
Ramos-Campo	Low	Low	N/A	Low	Low	N/A	Low	Low	6/6
Törpel (2020)	Low	Low	Low	High	Low	Low	Low	High	6/8
van Doorslaer de ten Ryen (2021)	Low	Low	N/A	Low	Low	N/A	Low	Low	6/6
Yan (2016)	Low	Low	N/A	N/A	Low	N/A	Low	Low	5/5

Low: Low risk of bias; High: High risk of bias; Unclear: unclear risk of bias. N/A: not applicable.

Table S5. Equations used for the calculation of effect sizes.

	H group versus N group	Post versus Pre training
Standardized mean change	$d = c(df_{H,N}) \cdot \left[\frac{(\bar{X}_{pre,H} - \bar{X}_{pos,H}) - (\bar{X}_{pre,N} - \bar{X}_{pos,N})}{\bar{S}_{pre}}\right]$	$d = c(df) \cdot \left[\frac{\bar{X}_{pre,H} - \bar{X}_{pos,H}}{S_{pre}}\right]$
Mean baseline standard deviation	$\bar{S}_{pre} = \sqrt{\frac{(n_H - 1) \cdot S^2_{pre,H} + (n_N - 1) \cdot S^2_{pre,N}}{n_H + n_N - 2}}$	
Correction factor	$c(df_{H,N}) = 1 - \frac{3}{4(n_H + n_N - 2) - 1}$	$c(df) = 1 - \left(\frac{3}{4(n - 1) - 1}\right)$
Variance[a]	$S^2(d) = [c(df_{H,N})]^2 \cdot 2(1-r) \cdot \left(\frac{n_H + n_N}{n_H n_N}\right) \cdot \left(\frac{n_H + n_N - 2}{n_H + n_N - 4}\right) \cdot \left[1 + \frac{n_H \cdot n_N \cdot d^2}{2(1-r)(n_H + n_N)}\right] - d^2$	$S^2(d) = [c(df)]_2 \cdot \left[\frac{2 \cdot (1-r)}{n}\right] \cdot \left(\frac{n-1}{n-3}\right) \cdot \left[1 + \frac{n \cdot d^2}{2 \cdot (1-r)}\right] - d^2$

= Normoxic group; H = Hypoxic group; d = standardized effect size; c(df) = correction factor; X = mean; $\bar{S}_{pre}$ = mean baseline standard deviation; n = sample size; S^2 = Variance; = 0.7, according to the standard r value recommended by Rosenthal [1].
Rosenthal R. Meta-analytic procedures for social research. Newbury Park, CA: Sage 1991.

Figure S1. R code for the statistical analysis and forest plot.

```
#forest plot (subanalysis example, severity of hypoxia in RM)
forest(res, xlim=c(-15, 6.5), ylim=c(-2, 38),
       at=(c(-2, 0, 2.5)),
       order = dat$Severity,
       header = FALSE,
       cex = 0.85,
       xlab="Standardized Mean Difference",
       rows=c(4:12, 17:34),
       slab = dat$Author,
       ilab=cbind(format(round(dat$aE,1)),
                  format(round(dat$n_E,1)),
                  format(round(dat$aC,1)),
                  format(round(dat$n_C,1)),
                  format(round(dat$aCambio,1)),
                  format(round(dat$Spre,1)),dat$weights),
       ilab.xpos=c(-8.5, -7.5, -6, -5, -3.5, -2.5, 3),
       mlab=mlabfun("RE Model for All Studies", res))

text(c(-8.5, -7.5, -6, -5, -3.5, -2.5, 3), 37, c("ΔH", "n",
"ΔN", "n", "ΔH-ΔN", "Spre", "Weight"))
text(c(-8,-5.5), 38, c("H group", "N group"))
text(-15, 37, "Study", pos=4)
text(4.5, 38, "Std. MD", pos=4)
text(6.5, 37, "Random [90%CI]", pos=2)
text(-15, 35, "Moderate (14.3 - 16 %FiO2)", pos=4, font = 4,
cex = 0.95)
```

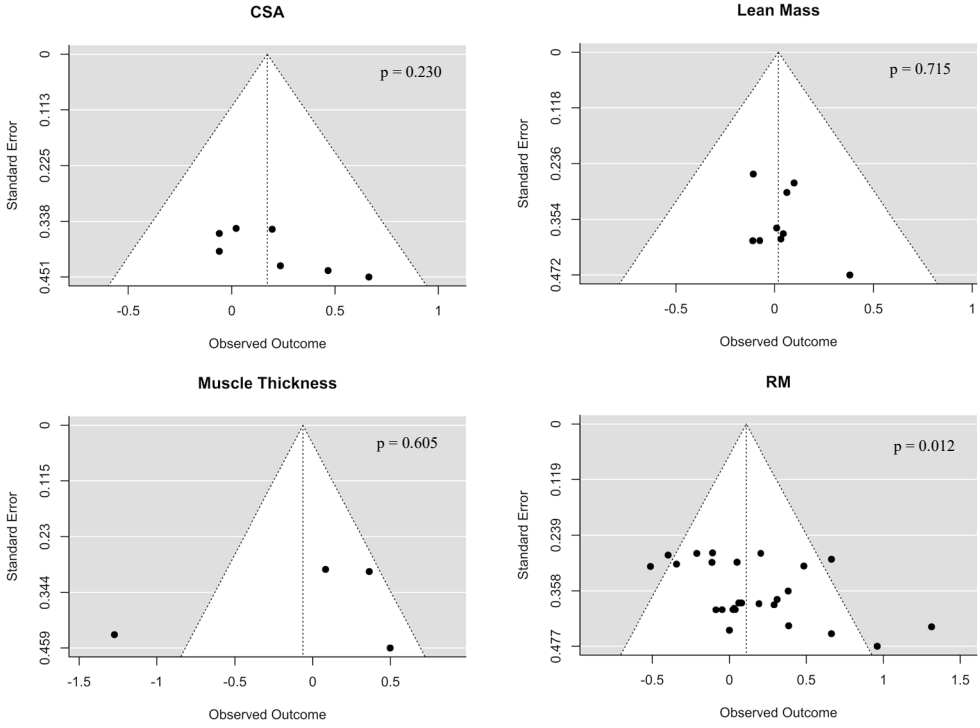
```
text(-15, 13, "High (<14.3 - 11 %FiO2)", pos=4, font = 4, cex
= 0.95)

#Use in case of multilevel analysis in subanalysis
res.m <- rma.mv(yi, vi, level=90, subset=(Severity=="Mid"),
random = ~ 1 | studyid/esid, data=dat)
res.h <- rma.mv(yi, vi, level=90, subset=(Severity=="High"),
random = ~ 1 | studyid/esid, data=dat)
I2m <- (res.m$sigma2[2])/(res.m$sigma2[1]+res.
m$sigma2[2]+mean(dat$vi, subset=(Severity=="Mid")))*100
I2h <- (res.h$sigma2[2])/(res.h$sigma2[1]+res.
h$sigma2[2]+mean(dat$vi, subset=(Severity=="High")))*100
mlabfunm <- function(text, res) {
  list(bquote(paste(.(text),
                    " Q = ", .(formatC(res$QE, digits=2,
format="f")),
                    ", df = ", .(res$k - res$p),
                    ", p ", .(metafor:::.pval(res$QEp,
digits=2, showeq=TRUE, sep=" ")), "; ",
                    I^2, " = ", .(formatC(I2m, digits=1,
format="f")), "% ")))}
mlabfunh <- function(text, res) {
  list(bquote(paste(.(text),
                    " Q = ", .(formatC(res$QE, digits=2,
format="f")),
                    ", df = ", .(res$k - res$p),
                    ", p ", .(metafor:::.pval(res$QEp,
digits=2, showeq=TRUE, sep=" ")), "; ",
                    I^2, " = ", .(formatC(I2h, digits=1,
format="f")), "% ")))}

addpoly(res.m, level=90, row=15.5, cex=0.85, efac=1.5,
width=c(4,5,4), mlab=mlabfunm("Heterogenity:", res.m))
addpoly(res.h, level=90, row= 2.5, cex=0.85, efac=1.5,
width=c(4,5,4), mlab=mlabfunh("Heterogenity:", res.h))
text(-15, 14.5, pos=4, cex=0.85, bquote(paste("Test for
overall effect: ",
                                              Z, " = ",
.(formatC(res.m$zval, digits=2, format="f")),
                                              ", p = ",
.(formatC(res.m$pval, digits=2, format="f")))))
text(-15, 1.5, pos=4, cex=0.85, bquote(paste("Test for
overall effect: ",
                                            Z, " = ",
.(formatC(res.h$zval, digits=2, format="f")),
```

```
                                                    ", p = ",
.(formatC(res.h$pval, digits=2, format="f")))))
text(-15, -2, pos=4, cex=0.85, bquote(paste("Test for overall
effect: ",
                                                  Z, " = ",
.(formatC(res$zval, digits=2, format="f")),
                                                    ", p = ",
.(formatC(res$pval, digits=2, format="f")))))
```

Figure S2. Funnel plot of the analyzed variables.



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