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Regulation of COVID19 receptor protein by vitamin D and exercise

Submission date	Recruitment status	[X] Prospectively registered
17/02/2024	No longer recruiting	[ ] Protocol
Registration date	**Overall study status**	[ ] Statistical analysis plan
26/02/2024	Completed	[ ] Results
Last Edited	**Condition category**	[ ] Individual participant data
26/02/2024	Other	[ ] Record updated in last year

Plain English summary of protocol

Background and study aims.
This research project aims to understand how a protein called angiotensin-converting enzyme 2 (ACE2) behaves in human skeletal muscle and the lining of the mouth when people engage in intense exercise. We also want to see if taking vitamin D3 supplements affects how ACE2 works. ACE2 is important for diseases like COVID-19 and other long-term health conditions. We think that ACE2 becomes more active when cells are stressed and when people take vitamin D3.

ACE2 does specific jobs in the body, like helping blood vessels relax and reducing inflammation. It's also used by the virus that causes COVID-19 to get into cells, especially in the lining of the mouth. Previous research suggests that women may have more ACE2 in their muscles than men. We've also seen that intense exercise can increase ACE2 levels in muscle.

To learn more, we plan to study 30 healthy young volunteers. We'll give them either a fake treatment or vitamin D3 supplements and then ask them to do intense exercise tests. We'll take samples from their mouth and muscles to see how ACE2 behaves. This matters because vitamin D3 levels seem to affect how severe COVID-19 can be. We'll also look at how well their muscles use oxygen to understand more about how ACE2 works.

Who can participate?
Healthy, physically active volunteers aged between 18 and 40 years old, of both sexes, non-smokers, not taking any medication or nutritional supplements, without medical contraindications to maximal exercise. In the case of females, not being pregnant. Basal 25 (OH) vitamin D concentration should be below 80 ng/mL. Volunteers must be able to perform exercise on a cycle ergometer (a static bike).

What does the study involve?
Participating in this study involves 5-6 visits to the laboratory for the assessment of body composition by dual-energy X-ray absorptiometry (DXA) and familiarization with the exercise protocol, which included low and maximal exercise intensity on a cycle ergometer, followed by a brief occlusion of the circulation. This is followed by a five-day supplementation phase, requiring an early visit to the laboratory on days 1, 3, and 5 for Vitamin D3 supplementation (25.000 IU) or a placebo ingested with 50 mL of juice. On day 5th, after the intake of Vit D3, a thin catheter will

be placed into the femoral vein with local anaesthesia and a muscle biopsy will be taken from one of the thighs, also under local anaesthesia. After that, the main experiment will be performed, as previously assayed in the laboratory, ending with a muscle biopsy taken from both legs. Before the start of the exercise and 5 minutes after the end of the exercise, the buccal cells will be collected with a special brush. The main experiment will be repeated after six weeks, ingesting Vitamin D3 or a placebo, so that all volunteers will have repeated the experiment one time with vitamin D3 and the other with a placebo in random order and without knowledge of the condition (double-blinded).

What are the possible benefits and risks of participating?
Participants in this study will have their health status assessed, as well as their body composition. Blood samples will be obtained before the start of the study as a part of basic health screening, and your basal levels of vitamin D3 will be assessed. During exercise, your VO2max and exercise capacity will be evaluated. Participants will receive medical advice if any anomaly is detected during the screening or exercise. The main risk associated with this study is the occurrence of cardiac arrhythmias during exercise, which is extremely rare even in healthy volunteers. Cardiac arrhythmias could lead to cardiac arrest and death. However, this risk is similar to when the exercise is performed outside the laboratory. Participating in the study may help to identify a hidden cardiac condition, and in case of a cardiac event, the laboratory is equipped to provide proper medical care. The muscle biopsies may be painful, and the surgical injury (5 mm incision) may leave a small scar. The skin area of the muscle biopsies should be maintained clean and covered to minimize the risk of infection. Swimming is prohibited during the next seven days after the muscle biopsy.

Where is the study run from?
The study is carried out at Laboratorio de Rendimiento Humano, Edificio de Educación Física, Campus Universitario de Tafira, at the University of Las Palmas de Gran Canaria, by a team with more than 25-year experience in these types of studies.

When is the study starting, and how long is it expected to run for?
October 2022 to December 2024

Who is funding the study?
This study is financed by Ministerio de Ciencia e Innovación (Spain).

Who is the main contact?
Jose A L Calbet, lopezcalbet@gmail.com

Contact information

Type(s)
Public, Scientific, Principal Investigator

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Additional identifiers

EudraCT/CTIS number
Nil known

IRAS number

ClinicalTrials.gov number
Nil known

Secondary identifying numbers
PID2021-125354OB-C21

Study information

Scientific Title
Regulation of ACE2 expression in human skeletal muscle by exercise and vitamin D in young males and females

Acronym
ACEVID

Study objectives
1. Vit D3 supplementation increases ACE2 expression in the cells of the oral mucosa and skeletal muscle.
2. Vit D3 supplementation improves mitochondrial respiration in human skeletal muscle.
3. Vit D3 supplementation attenuates exercise-induced ROS signalling.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 06/10/2022, Comité ético de investigación humana (Edificio de Educación Física, Campus Universitario de Tafira, Las Palmas de Gran Canaria, 35017, Spain; +34 928 451 030; sviit@ulpgc.es), ref: CEIH-2022-06

Study design
Randomized double-blind placebo-controlled crossover clinical trial

Primary study design
Interventional

Secondary study design
Randomised cross over trial

Study setting(s)
Laboratory

Study type(s)
Prevention, Efficacy

Participant information sheet
Not available in web format, please use contact details to request a participant information sheet.

Health condition(s) or problem(s) studied
Regulation of COVID19 receptor protein by vitamin D and exercise

Interventions
Thirty healthy young volunteers (15 males and 15 females) will be recruited to test the effects of Vit D3 dietary supplementation or placebo in oral mucosae and skeletal muscle ACE2 protein expression. Before administering supplements, a 10 mL resting blood sample will be obtained to determine their health status (general biochemistry and hemogram).

Two weeks later, the main experiments will start, comprising two supplementation trials (5 days) in a double-blind crossover design, including two conditions: placebo or Vit D supplementation. On each trial, subjects will report to the laboratory at 7:00 a.m. and after the ingestion of the last dose of a placebo or Vit D3 (assigned randomly using an online tool). The corresponding supplement (placebo or Vit D3) will be administered in 3 doses of 25000 IU of Vit D3 on alternate days for 5 days (i.e., days 1, 3, and 5). On the 5th day in the morning, a 20 mL blood sample will be obtained from the femoral vein after ingesting the corresponding supplement. This will be followed by a collection of buccal mucosae cells with a cytobrush, and a muscle biopsy will be taken from the musculus vastus lateralis with the Bergstrom technique. The baseline sprint capacity (10 s all-out), VO2max, maximum fat oxidation capacity (indirect calorimetry) and muscle O2 extraction capacity (near-infrared spectroscopy, NIRS) will be assessed. Subjects will perform incremental exercise to exhaustion, followed by 20 seconds of ischaemia, a final 10 s all-out sprint, immediately followed by 1 minute of ischaemia applied in only one leg. At the end of the ischaemia, a bilateral muscle biopsy (occluded and non-occluded leg) will be obtained at the end of the exercise test performed with supplements, followed five min later by the collection of a 20 mL blood sample and ten minutes later by a collection of mucosal cells. In pre-and post-exercise blood samples, the circulating levels of 25(OH)D, SHBG, total testosterone, oestradiol, cortisol, soluble ACE2, Ang II, Ang 1-7, and miRNAs will be assessed.

Six weeks later, the whole experiment will be repeated after administering the alternative treatment, i.e., placebo, to the subjects that received Vit D3 in the first trial and vice versa. In the muscle samples, mitochondrial respiration (high-resolution respirometry) and the protein expression levels of ACE2 and main stress kinases will be assessed. The plasma levels of 25(OH)D will also be determined, as well as femoral vein blood gases, electrolytes, acid-base balance and vasoactive peptides.

Intervention Type
Supplement

Primary outcome measure
Skeletal muscle protein expression levels of ACE2 measured using western blotting at baseline and at the end of the exercise.

Secondary outcome measures
1. Buccal mucosae protein expression levels of ACE2 measured using western blotting at baseline and at the end of exercise.
2. Skeletal muscle ROS-induced signalling measured using western blotting at baseline and at the end of exercise.
3. Exercise performance (power output) measured using a cycle ergometer, at baseline, during an incremental exercise to exhaustion and during a 10 s sprint
4. Exercise VO2 measured using a metabolic cart, at baseline and during an incremental exercise to exhaustion.
5. Maximal fat oxidation measured using a metabolic cart, during an incremental exercise to exhaustion.
6. Muscle mitochondrial maximal VO2 measured ex vivo measured using high-resolution respirometry at baseline.
7. Skeletal muscle O2 extraction capacity measured using near-infrared spectroscopy, at rest and during incremental exercise to exhaustion.
8. Femoral vein blood gases measured using an intravenous catheter and blood gas analyser at rest and at the end of exercise.
9. Femoral vein acid-base balance measured using an intravenous catheter and blood gas analyser at rest and at the end of exercise.
10. Femoral vein electrolytes measured using an intravenous catheter and blood gas analyser at rest and at the end of exercise.
11. Femoral vein vasoactive peptides measured using ELISA at baseline and after exercise.

Overall study start date
06/10/2022

Completion date
31/12/2024

Eligibility

Key inclusion criteria
1. Males and females between 18 and 40 years old
2. Physically active, but not submitted to periodized training.

Participant type(s)
Healthy volunteer

Age group
Adult

Lower age limit
18 Years

Upper age limit
40 Years

Sex
Both

Target number of participants
30

Key exclusion criteria
1. Medical contraindications for maximal exercise
2. Smoking
3. Taking drugs or medications
4. Taking supplements
5. Fainting when seeing blood
6. Not being able to pedal
7. Carrying orthopaedic prosthesis
8. Pregnancy
9. Basal 25 (OH) vitamin D concentration above 80 ng/mL

Date of first enrolment
01/03/2024

Date of final enrolment
01/10/2024

Locations

Countries of recruitment
Spain

Study participating centre
Laboratorio de Rendimiento Humano (IUIBS)
Edificio de Educación Física
Campus Universitario de Tafira
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Organisation
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Sponsor type
University/education

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ROR
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Funder(s)

Funder type
Government

Funder Name
Ministerio de Ciencia e Innovación

Alternative Name(s)
CienciaGob, Ministerio de Ciencia e Innovación de España, Ministry of Science and Innovation, Spanish Ministry of Science and Innovation, Ministry of Science and Innovation of Spain, Spain, Ministry for Science and Innovation, Ministeri de Ciència i Innovació, MCIN, MICINN

Funding Body Type
Government organisation

Funding Body Subtype
National government

Location
Spain

Results and Publications

Publication and dissemination plan
The main results of this study will be published in high-impact peer-reviewed journals.

Intention to publish date
01/03/2025

Individual participant data (IPD) sharing plan

The dataset generated and/or analysed during the current study will be available upon request from Jose A L Calbet (lopezcalbet@gmail.com). Only data dat can not be linked to the identity of participants will be facilitated and upon a sound request for research purposes.

IPD sharing plan summary

Available on request