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Detection of enhanced hormonal production in male athletes

Submission date
11/05/2019

Recruitment status
No longer recruiting

Registration date
15/05/2019

Overall study status
Completed

Last Edited
15/05/2019

Condition category
Other

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol

Background and study aims
Overtraining syndrome (OTS) is a syndrome that affects the majority of elite athletes at least once in their lifetime, and leads to a reduction in the sports performance associated with fatigue, which is not explained by any condition. In the top of this, OTS is extremely challenging to recover from, and may interrupt many athletes' careers. Initially alleged to be caused by excessive training, researchers noticed that this syndrome still occurred even when training was not excessive. Also, researchers failed to discover markers and the baseline causes for OTS. The aim of this study is to find new biomarkers and risk factors for OTS, in order to detect athletes at high risk for OTS and prevent it. For this, athletes affected by OTS are compared to similar healthy athletes and also to healthy sedentary volunteers.

Who can participate?
Male athletes between 18 and 50 years old, without any known disorder, and healthy sedentary volunteers

What does the study involve?
Participants undergo blood tests, evaluation of sleep, psychological, social and eating patterns, and analysis of body metabolism and composition, with a maximum interval from the beginning to the end of the tests of 10 days.

What are the possible benefits and risks of participating?
The benefits for the participants include an individual and personalised report from all tests performed, including a thorough interpretation for each of the results. The study will likely add a lot to the currently existing knowledge in the field of Sports Medicine.

Where is the study run from?
Federal University of São Paulo (Brazil)

When is the study starting and how long is it expected to run for?
October 2016 to May 2017.

Who is funding the study?
Investigator initiated and funded

Who is the main contact?
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Contact information

Type(s)
Scientific

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Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
EROS1111

Study information

Scientific Title
Enhancement of hypothalamic-pituitary activity in male athletes: a novel hormonal mechanism of physical conditioning

Acronym
Endocrine and Metabolic Responses on Overtraining Syndrome (EROS)

Study objectives
Identify novel mechanisms of hormonal conditioning in athletes.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 04/06/2015, Ethical committee of the Federal University of São Paulo (Rua Francisco de Castro nº 55, Vila Clementino, CEP 04020-050 – São Paulo/SP; Tel: +55 (0)11 5571 1062 or +55 (0)11 5539 7162; Email: cep@unifesp.br), approval number: 1093965

Study design
Observational case-control study

Primary study design
Observational

Study type(s)
Diagnostic

Health condition(s) or problem(s) studied
Overtraining syndrome

Interventions
A preliminary analysis of the candidates was done by email correspondence, and included questions regarding age, sex, and approximate body weight and height. Based on the candidate' s responses, their approximate body mass index (BMI) was calculated. If no exclusion criteria were identified, an individual interview was then scheduled. At the interview, body weight and height were verified using high precision weight and height scales. Questions regarding other conditions, use of medications or hormones, and characteristics of the sport (in the case of athletes) were also asked, and age was confirmed by verification of an identity card. The quantification of training load was recorded by the coach of each athlete, but not in a systematic way, as the recruitment occurred transversally in order to collect real-life training data (i.e., it was not controlled). The researchers required a minimum amount of physical activity for potential exercise-induced adaptions.

Candidates who fulfilled criteria were selected. After signing a written consent, the remaining subjects underwent biochemical examination to exclude confounding disorders and prevent inclusion of subjects with altered basal and stimulated hormone levels due to inflammation, infection, kidney disease, lipid metabolism abnormalities, vitamin deficiencies, or obvious hormonal dysfunctions. In ATL, exams were performed from 36 to 48h after the last training session.

After the selection process, the subjects underwent basal biochemical tests, hormonal responses to stimulation tests, and evaluation of sleep, psychological, social and eating patterns, and analysis of the body metabolism and composition, as part of the different arms of the EROS study, with a maximum interval from the beginning to the end of the tests of 10 days.

For the CST, subjects were required to fast for 8 hours, and to arrive at the laboratory at 7:30h in the morning. They sat in blood-drawing chairs and rested for 30 min. Ten mL of blood (divided into two EDTA tubes) was collected before and 30 and 60 minutes after an intravenous administration of 250 µg of cosyntropin (as recommended by the guidelines of Endocrinology societies) for analysis of cortisol.

Subjects underwent the ITT 48 hours after the CST, following an 8-hour fasting. Subjects arrived at the laboratory at 7:30h in the morning, were seated in a blood-drawing chair, and rested for 30 min. Then, 0.1 IU/kg of regular insulin was administered intravenously after blood collection (10 mL in EDTA and plasma tubes) at time zero (baseline). Capillary glucose was checked every 5 min from time 10 min after insulin administration, or whenever subjects reported symptoms. Blood for time one was collected when: 1) capillary glucose was <30 mg/dL without symptoms; 2) subjects classified symptoms of hypoglycemia as moderate to severe (5–10) regarding either adrenergic (shakiness, cold sweating, heart palpitations, or pallor) or neuroglycopenic (sleepiness, mood changes, or unrest) symptoms, or both; or 3) if capillary glucose was <45 mg /dL in the presence of any symptom. If after 40 min none of these three criteria was achieved, an extra 0.05 IU/kg of regular insulin was administered intravenously; and again after an additional 40 min, if none of the criteria was achieved. Finally, if hypoglycemia did not occur, the subject would be withdrawn from the study due to likely insulin resistance (which makes it unfeasible to perform a proper ITT test). However, none of the patients required a third dose of insulin.

After time one blood collection, 10 mL of 50% glucose solution was infused intravenously and high-glycemic index and pure carbohydrate food (lemon or strawberry dairy-free sorbet, Diletto, Brazil) was offered ad libitum. Ten mL of blood was collected again, 30 min after the hypoglycemic episode (time two). In all blood samples cortisol, ACTH, GH, prolactin and glucose were determined, as well as the absolute ACTH/cortisol ratio at all times during ITT24-26. During the ITT the time-to-hypoglycemia (min) since insulin administration and self-reported intensity of adrenergic and neuroglycopenic symptoms were evaluated on a scale of 0 to 10 (0=asymptomatic; 10=severe symptoms). The researchers did not perform the 60 min for all athletes, as the first round (a “pilot” evaluation, with three sedentary and three healthy athletes) did not disclose differences for any of the hormones between 30 and 60 minutes after hypoglycemia. Also, protocols for ITT admit variations regarding time for the blood collection, and whether the time for blood collect depended on the hypoglycemic episode or not.

Due to the risk of severe hypoglycemia during ITT, subcutaneous glucagon pens were always available (GlucaGen HypoKit, 1 µg, Novo Nordisk), as well as 20 mL-syringes containing 50% glucose solution and an automated external defibrillator (AED).

Basal and hypoglycemia-induced serum cortisol, plasma ACTH, serum GH and serum prolactin levels were determined by commercially available electrochemiluminescence assays that were previously validated, standardized and tested. The detection limits for ACTH and GH were 5.0 pg /mL and 0.05 g/L, respectively, but there was no minimal analytical limit for the other markers. The intra- and inter-assay coefficients of variability of all the biochemical markers measured in all arms of the EROS study were below 3.0% and 3.5%, respectively.

The researchers evaluated basal and hypoglycemia-induced and absolute changes in the levels of cortisol, ACTH and prolactin during the ITT (from time zero to time two); GH changes were not determined because of its wide pulse amplitude. Mean time-to-hypoglycemia and intensity of adrenergic and neuroglycopenic symptoms were also compared between groups.

All tests and the collection of blood for analysis were medically supervised. Blood or plasma collection tubes were checked before and after each collection to ensure that an appropriate tube type was used for the biomarker and that each subject was properly identified. Following collection, the tubes were immediately centrifuged or analyzed to prevent loss of quality of the collected material. For the present study, the researchers analyzed the hormonal responses to stimulation tests.

Intervention Type

Other

Primary outcome(s)

EROS-HPA axis:
1. ACTH and cortisol measured using commercially available electrochemiluminescence assay: basal, during hypoglycemia, 30 min after hypoglycemia, and during an insulin tolerance test (ITT) on Day 2 (48h)
2. Cortisol response to a cosyntropin stimulation test (CST) measured using commercially available electrochemiluminescence assay at 30 min and 60 min after injection on Day 1 (24h)
3. Salivary cortisol rhythm (SCR): salivary cortisol measured using commercially available electrochemiluminescence assay at awakening, 30 min after, at 4PM, at 11PM, cortisol awakening response (CAR), on Day 0 (baseline)

EROS-STRESS:
1. GH and prolactin measured using commercially available electrochemiluminescence assay: basal, during hypoglycemia, 30 min after hypoglycemia, prolactin increase during ITT, on Day 2 (48h)
2. Glucose behaviour during an ITT: serum glucose measured using enzymatic assay of hexokinase, basal and during hypoglycemia, adrenergic symptoms during hypoglycemia (0-10), neuroglycopenic symptoms during hypoglycemia (0-10), on Day 2 (48h)

EROS-BASAL:
1. Hormonal markers measured on Day 1 (24h):
1.1. Total testosterone measured using chemiluminescence assay
1.2. Estradiol measured using chemiluminescence assay
1.3. IGF-1 measured using chemiluminescence assay
1.4. TSH measured using chemiluminescence assay
1.5. Free T3 measured using chemiluminescence assay
1.6. Total catecholamines measured using calorimetric enzymatic assays
1.7. Metanephrines measured using calorimetric enzymatic assays
1.8. Noradrenaline measured using calorimetric enzymatic assays
1.9. Epinephrine measured using calorimetric enzymatic assays
1.10. Dopamine measured using calorimetric enzymatic assays
1.11. Metanephrines measured using calorimetric enzymatic assays
1.12. Normetanephrines measured using calorimetric enzymatic assays
2. Biochemical markers measured on Day 1 (24h) :
2.1. Erythrocyte sedimentation rate (ESR) measured using automated spontaneous sedimentation method
2.2. Hematocrit measured using automated assay
2.3. C-reactive protein measured using latex-intensified immunoturbidimetry
2.4. Lactate measured using enzymatic assays
2.5. Vitamin B12 measured using chemiluminescence assay
2.6. Ferritin measured using chemiluminescence assay
2.7. Neutrophils measured using automated assay
2.8. Lymphocytes measured using automated assay
2.9. Eosinophils measured using automated assay
2.10. Creatine kinase measured using calorimetric activity assays
3. Ratios measured on Day 1 (24h):
3.1. Testosterone-to-estradiol ratio measured using the calculation of the ratio between total testosterone [chemiluminescence assay] and estradiol [chemiluminescence assay]
3.2. Testosterone-to-cortisol ratios measured using the calculation of the ratio between total testosterone [chemiluminescence assay] and cortisol [chemiluminescence assay]

3.3. Neutrophil-to-lymphocyte measured using the calculation of the ratio between neutrophils [automated assay] and lymphocytes [automated assay]
3.4. Platelet-to-lymphocyte ratios measured using the calculation of the ratio between platelets [automated assay] and lymphocytes [automated assay]

EROS-PROFILE:
1. General patterns measured using a person to person interview on Day 0 (baseline):
1.1. Duration of night sleep (h/night)
1.2. Self-reported sleep quality (0–10)
1.3. Self-reported libido (0–10)
1.4. Number of hours of activities besides professional training
2. Eating patterns measured using a one-week food and nutrition record with manual calculation of mean intakes on Day 0 (baseline):
2.1. Calorie intake
2.2. Carbohydrate intake
2.3. Protein intake
2.4. Fat intake
3. Psychological patterns measured on Day 0 (baseline):
3.1. Profile of Mood State (POMS) questionnaire (total score: -32 to +120)
3.2. Anger subscale (0 to 48)
3.3. Confusion subscale (0 to 28)
3.4. Depression subscale (0 to 60)
3.5. Vigour subscale (0 to 32)
3.6. Fatigue subscale (0 to 28),
3.7. Tension subscale (0 to 36)
4. Body metabolism measured using indirect calorimetry on Day 0 (baseline):
4.1. Measured-to-predicted basal metabolic rate (BMR, %)
4.2. Percentage of fat burning compared to total BMR (%)
5. Body composition measured using electrical bioimpedance (InBody770, Biospace, South Korea) and air displacement plethysmograph (Bod Pod, CosMed, USA) at Day 0 (baseline):
5.1. Body fat percentage
5.2. Muscle mass weight
5.3. Body water percentage
5.4. Extracellular water compared to total BW
5.5. Visceral fat
5.6. Waist circumference
5.7. Chest-to-waist circumference ratio

Key secondary outcome(s))
There are no secondary outcome measures

Completion date
30/06/2017

Eligibility

Key inclusion criteria
All participants:
1. Male
2. Aged between 18 and 50 years
3. BMI between 20.0 and 32.9 kg/m2

4. Absence of previous psychiatric disorders and use of centrally-acting drugs
5. Absence of any hormonal therapy in the previous six months

Healthy elite athletes (ATL):
The following additional inclusion criteria regarding training aspects were required from all ATL regarding training level:
1. Exercise at least four times a week, for a total of >300 min a week
2. Moderate-to-vigorous training intensity (self-perception comparing own training with that of others, based on the Talk Test)
3. Continuous training for the current sport(s) for at least six months, without interruption for >30 days

Non-physically active control subjects (NPAC) were required to:
1. Fulfill the initial inclusion criteria
2. Be sedentary (without any physical activity, including light exercises) for at least three years
3. No history of exercise that would fulfill the criteria for ATL

Participant type(s)
Mixed

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 years

Sex
Male

Key exclusion criteria
Does not meet the inclusion criteria

Date of first enrolment
01/10/2016

Date of final enrolment
30/11/2016

Locations

Countries of recruitment
Brazil

Study participating centre
Federal University of São Paulo
Rua Pedro de Toledo, 781, 13th Floor

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04215050

Sponsor information

Organisation
Federal University of São Paulo

ROR
https://ror.org/02k5swt12

Funder(s)

Funder type
Other

Funder Name
Investigator initiated and funded

Results and Publications

Individual participant data (IPD) sharing plan
The researchers stored the full methodology, the raw results of each participant, which is entirely anonymous, and the raw statistical analysis. The weblink for the raw data is: https://osf.io/bhpq9/, which is promptly available upon accessing, and ever available. Written consent from participants was obtained, and there were no ethical restrictions.

IPD sharing plan summary
Stored in repository

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article	results	01/07/2018	14/05/2019	Yes	No
Results article	results	01/08/2018	14/05/2019	Yes	No
Study website	Study website	11/11/2025	11/11/2025	No	Yes