RESEARCH PROTOCOL

The Effectiveness and Safety of High Intensity Interval Training Based on Oxidative Stress and Inflammatory Markers in The Management of Overweight Male Subjects

NCT Number/ID : Not Assigned Yet

Protocol ID : 20-09-1081

Document Date October 8th, 2021

General Rules

1. Attendance scheduling is done 7 days in advance and then sent to IMERI's secretariat
2. Office hours are 09.00 – 15.00 on Monday – Friday. If the subject is unable to attend at office hours, he will be directed to attend after 15:00. If the subject is unable to attend on weekdays, he will be directed to attend on Saturdays with office hours 09.00 – 12.00
3. The presence of the subjects is not scheduled to coincide with each other
4. The number of research teams present is at least 2 people
5. D-1 presence, research team must:
    a. inform the subject to attend 30 minutes before the session start and come alone without bringing any other unauthorized parties.
    b. inform the subject to bring comfortable sports clothes and shoes, towels, spare surgical masks, face shields, hand sanitizer and own drinking bottles.
    c. submit an online covid-19 self-assessment form and ensure the subject fills it out immediately.
    d. ensure the subjects do not use public transport modes, except taxis
6. On D-day:
    a. ensure that the room and equipment have been cleaned and disinfected as scheduled
    b. before entering the training room, COVID-19 safety protocols were carried out on both the research teams and subjects such as temperature checks, hand washing, changing clothes, disinfecting/changing shoes, and wearing new surgical masks.
    c. the subject waits in the designated room by always keeping a distance of 2 meters before it is declared that the activity can begin.
    d. during the activity, the research team used personal protective equipment, wear surgical masks, and perform hand washing before and after the examination. The research teams always wear gloves when holding a tool or subject.
    e. during the activity, it is attempted to maintain the maximum distance for the research teams as well as the research subjects.
    f. after the activity is complete, the research team schedules for the next meeting. Subjects are advised to return home immediately while being reminded to protect themselves during the journey to home.

B. Baseline Assessment

1. D-1:
    a. the research team submits a PAR-Q form online and ensures the subject fills it out immediately. The subjects who came were those who were declared healthy and ready for examination.
    b. the research team briefly informs the purpose and method of examination. Subjects were advised 24 hours in advance not to do vigorous physical exercise and avoid taking heavy meals, caffeine, alcohol and cigarettes three hours before the examination
2. On D-day, the research teams conducted the following activities:
    a. explain informed consent and ensure the subject gives signature
    b. submit a research screening form to the subject to fill out on his own
    c. perform vital signs examination: blood pressure, temperature, pulse rate, frequency of breath. Write the results into the research screening form
    d. perform height measurement and write the results into a research questionnaire
    e. insert subject data into a laptop for body composition examination
    f. perform a body composition examination and fill out the logbook
    g. perform musculoskeletal examination
    h. assess the subject's screening form and put a signature
    i. if the subject is not used to doing treadmill before, then the subject is directed to familiarize treadmill by using modified Bruce protocol.
    j. checking the readiness of the CPET examination room. Subjects remain waiting in the BIA examination room when the room is not ready
    k. performs the installation of a polar monitor on the chest for *heart rate* (HR) monitoring throughout the CPET examination.
    l. performs a CPET examination using modified Bruce protocol
    m. enter the data of VO_2 Max examination results
    n. check the completeness of the data in the questionnaire and put a signature

C. Blood Sampling and Exercise Familiarization

1. D-1:

- the research team briefly informs the purpose and method of examination.
- the day before the blood collection, subjects were required to fast 12 hours before blood collection.
- subjects were advised 24 hours in advance not to do vigorous physical exercise, consume enough water, as well as avoid consumption of heavy foods and caffeine three hours before the examination.

2. On D-day, the research team conducted the following activities:
   - Blood Sampling
     i. Blood collection is done by installing a needle connected to the vacutainer in the blood vessels in the forearm and then taken blood as much as ± 8 cc. Previously applied alcohol 70% as an antiseptic action in the area of blood collection.
     ii. Blood collection is done by nurses who are used to taking blood.
   - Cognitive Examination
     - 1. Montreal Cognitive Assessment (MoCA)
     - 2. Trail Making Test (TMT) A dan B
     - 3. N-Back

     Subjects will receive one familiarization session before cognitive examination
   - Familiarization exercises
     i. Preparing the completeness and training facilities: cones, Borg scale banners, road signs and exercise monitoring forms
     ii. Fill out the exercise monitoring form with subject data.
     iii. Install a polar monitor on the chest for heart rate (HR) monitoring throughout the exercise and connect it to the research team's device for monitoring and data storage
     iv. Perform vital signs: blood pressure, pulse rate. Write the results into an exercise monitoring form
     v. Lead the exercises

     Warming up:
     - Stretching : stretching of shoulder muscles, pelvic muscles, and limb muscles
     - Jogging : 3-5 minutes
     - Wind sprint : 2-3 times sprint with a distance of 20 meters

- Rest : 2-3 minutes to lower pulse

Core exercise:

- Run back and forth with a distance of 4 meters 3 x 8 seconds
- Star jump 3 x 8 seconds
- Zig-zag running back and forth with a distance of 4 meters 3 x 8 seconds
- Burpees 3 x 8 seconds
- Run box shape with a distance of 2 meters 3 x 8 seconds

Rest between reps 25 seconds, during rest the subject remains actively moving

Rest between movements 2 minutes

Cooling down:

- Jogging : 3-5 minutes
- Stretching : stretching of shoulder muscles, pelvic muscles, and limb muscles
- HR and RPE are monitored at minutes 1, 3, 4, 5 and 15. Results are recorded in the exercise monitoring form

vi. After the exercise:

- Participants fill out the Physical Activity Enjoyment Scale / PAES independently
- The research team checked the completeness of the data and put a signature on the exercise monitoring form

- Explaining Bouchard's 3-day activity record and 3-day food record
- Explaining how to use Polar HR monitor and its smart-phone application, also how to take notes in online exercise recording forms.
- Sending Bouchard's 3-day activity record and 3-day food record to subjects

D. Offline and online training sessions:

a. The mechanism is the same as the familiarization of the exercise

b. At the first training session in week 1, 6 and 12, there will be serial blood test for fat burning effect (FGF21 and Irisin): before training, soon after training finished (0 hour), 1 hour after training (1 hour) and 3 hour after training (3 hour).

c. At the first training session in week 1 and the last training session in 12, there will be cognitive examination at 0 minute, 30 minute and 60 minute after training
d. Online training sessions will be supervised via video conference app. Research assistants will provide the instruction and participants will note the HR and RPE scale

E. Endline masurement:

- D-1:
    a. the research team briefly informs the purpose and method of examination.
    b. the day before the blood collection, subjects were required to fast 12 hours before blood collection.
    c. subjects were advised 24 hours in advance not to do vigorous physical exercise, consume enough water, as well as avoid consumption of heavy foods and caffeine three hours before the examination.
- On D-day, the research team conducted the blood sampling
    a. perform vital signs examination: blood pressure, temperature, pulse rate, frequency of breath. Write the results into the research screening form
    b. perform a body composition examination and fill out the logbook
    c. performs a CPET examination using modified Bruce protocol
    d. Blood collection is done by installing a needle connected to the vacutainer in the blood vessels in the forearm and then taken blood as much as ± 8 cc. Previously applied alcohol 70% as an antiseptic action in the area of blood collection.
    e. Blood collection is done by nurses who are used to taking blood 1 day after body composition and CPET measurements

F. Statistical Analysis Plan:

- Data analysis will be performed using licensed version of SPSS software from Universitas Indonesia.
- In univariate analysis, all categorical data will be presented as proportion and numeric will be presented as mean and standard deviations (if normally distributed) or median and minimum-maximum range (if not normally distributed).
- In bivariate analysis, Paired t-test will be used to identify the difference before and after the

intervention (if the data are normally distributed) or Wilcoxon test (if not normally distributed)

Me, **dr.** ***Risky Dwi Rahayu/dr. Retnoningtyas/dr. Ferdinandus Adri Pradhana /IKO*** Research Team led by ***Dr. dr. Nani Cahyani Sudarsono, Sp.KO*** from the **Sports Medicine Study Program, Faculty of Medicine, Universitas Indonesia** will conduct a study titled **The Effectiveness and Safety of High Intensity Interval Training Based on Oxidative Stress and Inflammatory Markers in The Management of Overweight Male Subjects**. The research was sponsored by **Universitas Indonesia.**

I will inform you about this research and invite you to be part of this research.

You can give consent to participate in this research by signing this form and you can freely withdraw from this research at any time. If you refuse to participate or withdraw from this study, the decision will not affect your relationship with me.

If you do not understand each statement in this form, you may ask me.

1. **Research objectives**

   We want to know how the safety level of high intensity interval training (HIIT) in overweight men. To find out we have to collect data and take samples for laboratory tests.

2. **Participation in research**

   Overall, this study will run for 3 months. If you decide to participate in this research, you will be asked for your willingness to follow our schedule and ensure that you can comply with the schedule. This research will involve you in several interview sessions, examinations and physical exercises. Each interview session will be conducted for approximately 1 to 2 hours, the examination session will approximately last for 4 hours and the physical training session will approximately last for 1 hour.

3. Reasons for choosing you

You were chosen because you fall into the criteria of selecting subjects, namely young adult men (18-30 years old) who are overweight and do not do physical exercise for the last 6 months or do physical exercise less than 150 minutes of aerobic physical activity according to WHO's recommendation. In addition, you do not smoke, have no history of cardiovascular or metabolic disease, and have no history of musculoskeletal injury in the last 1 year

4. Research procedures

4a. <u>Covid Vigilance Procedure</u>

This study implemented the Covid-19 vigilance protocol with the following details:

1. Researchers and subjects fill out a health risk assessment form the day before attending the research place.
2. Researchers and subjects are required to wear surgical masks, wash their hands, spray shoes with disinfectant, report themselves at the reception desk and change clothes before entering the study site. The use of research site facilities is regulated by officers.
3. Researchers are required to use face shields and surgical masks when conducting assessments at the training site.
4. The number of researchers and subjects is adjusted to the maximum capacity of the room at the research site to ensure physical distancing.
5. Maintain distance between researchers and subjects, speaking in a safe distance and only when needed.
6. Cleaning work equipment and exercise equipment after use as well as washing hands with soap and running water or hand sanitizer.
7. Change clothes and take a shower after arriving home.

4b. Drug Information or Intervention Procedures

Describe information about the drugs studied or intervention procedures that will be performed during this study. Describe the sample to be taken, the volume, also include the period of participation of the study subject and the number of times the treatment /

sampling.

1. You will be interviewed by a doctor to ask: Name, age, history of disease, history of injury, habits of physical activity, intake of nutrients.
2. Undergo a physical examination by a doctor to check the health status, body composition, heart-lung ability.
3. The night before the study you were asked to fast, but it is permissible to drink water as necessary.
4. On the day of the start of the study, you are asked to come at the specified time for further blood collection.
5. Blood sampling will be done 2 times during the research period. Blood collection is done by installing a needle on the blood vessels in the forearm and then taken blood as much as $\pm$ 8 cc. Previously applied alcohol 70% as an antiseptic action in the area of blood collection. The first blood collection was done before the physical exercise intervention, while the 2nd blood collection was done after the 12th week of the study.

   This blood collection aims for laboratory examination of fat profiles in the form of HDL, LDL, triglycerides and total cholesterol as well as biological markers of oxidative stress, namely superoxide dismutase (SOD) and malondialdehyde (MDA) and also inflammatory markers, namely Interleukin-6 (IL-6) and tumor necrosing factor (TNF-alpha)
6. Blood collection is done by nurses who are used to taking blood.
7. On the specified day of physical exercise, you will get supervision by trainers and doctors to assess the effectiveness and safety of the exercise. During and after the physical training session will be examined to determine the level of intensity and comfort of the exercise.

5. Risks, side effects and procedures

The physical exercises you follow will always get supervision from trainers and doctors. In case of injury during physical exercise, the doctor will perform the first and follow-up treatment in accordance with clinically tested procedures.

6. Benefits

The benefit you can get is getting physical exercise with direct supervision from a doctor as well as physical and laboratory examinations to find out the body composition and state of blood for free.

7. Compensation

You will get a transportation change of Rp 50.000,- for every arrival to this research place.

8. Financing

The financing of this research will be fully borne by the research team through the assistance of research grants from the University of Indonesia International Indexed Publication Q2 (PUTI Q2) 2020.

9. Confidentiality

All data collected in this study will be kept confidential and cannot be accessed without the permission of the research team. Presentation of research results in scientific meetings / conferences and publications in scientific journals will not include your name. However, representatives of sponsors, ethics committees, and national authorities governing drug use will have access to research data for verification.

10. Obligations of research subjects

As the subject of the study, you are obliged to follow the rules or research instructions as written above. If something is not clear, you can ask further questions to the research team.

11. Right to refuse and resign

You don't have to participate in this study if you don't want to. You must understand that even if you agree to participate, you have the right to withdraw from this research. If you refuse to participate or withdraw from this study, the decision will not affect your relationship with me and will have no impact on any of these. I will give you the

opportunity at the end of this explanation to be able to consider the decision to be taken.

12. Additional Information

You are given the opportunity to ask all the things that are not yet clear in relation to this study. If you need further explanation at any time, you can contact **Dr. dr. Nani Cahyani, Sp.KO (0816736605)/dr. Risky Dwi Rahayu (08124984035).**

PARTICIPATION APPROVAL SHEET

All these explanations have been submitted to me and all my questions have been answered by a team of researchers/doctors. I understand that if I need an explanation, I can ask Dr. dr. Nani Cahyani, Sp. KO or dr. Risky Dwi Rahayu.

Certificate of Approval (*Consent*)	
I have read all the explanations about this research. I have been given the opportunity to ask and all my questions have been answered clearly. I am willing to participate in this research study voluntarily. Subject/guardian name _____ Study participants' signatures _____ Date_____ day/month/year	I confirm that participants have been given the opportunity to ask about this study, and all questions have been answered correctly. I confirm that consent has been given voluntarily. Name of researcher/approver _____ Researcher signature/approval requester Date _____ day/month/year

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