Cryobiology xxx (2015) 1–7

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

The whole body cryostimulation modifies irisin concentration and reduces inflammation in middle aged, obese men

Katarzyna Dulian [a], Radosław Laskowski [b], Tomasz Grzywacz [c], Sylwester Kujach [b], Damian J. Flis [d], Mirosław Smaruj [e], Ewa Ziemann [f, *]

[a] Gdansk University of Physical Education and Sport, Department of Physiotherapy, Kazimierza Gorskiego 1, 80-336 Gdansk, Poland
[b] Gdansk University of Physical Education and Sport, Department of Physiology, Kazimierza Gorskiego 1, 80-336 Gdansk, Poland
[c] Institute of Sport, Department of Physiology, Trylogii 2/16, 01-982 Warsaw, Poland
[d] Medical University of Gdansk, Department of Bioenergetics and Physiology of Exercise, Marii Skłodowskiej-Curie 3A, 80-210 Gdansk, Poland
[e] Gdansk University of Physical Education and Sport, Department of the Theory of Sport, Kazimierza Gorskiego 1, 80-336 Gdansk, Poland
[f] Gdansk University of Physical Education and Sport, Department of Physiology and Pharmacology, Kazimierza Gorskiego 1, 80-336 Gdansk, Poland

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form
15 August 2015
Accepted 11 October 2015
Available online xxx

Keywords:
Cold
Hepcidin
Iron
Cardiorespiratory fitness level
Fat tissue
Irisin

ABSTRACT

The anti-inflammatory effect induced by exposure to low temperature might trigger the endocrine function of muscle and fat tissue. Thus, the aim of this study was to investigate the influence of the whole body cryostimulation (CRY) on irisin, a myokine which activates oxygen consumption in fat cells as well as thermogenesis. In addition, the relationship between hepcidin (Hpc) – hormone regulating iron metabolism, and inflammation was studied.

A group of middle aged men (n = 12, 38 ± 9 years old, BMI > 30 kg m^{-2}) participated in the study. Subjects were exposed to a series of 10 sessions in a cryogenic chamber (once a day at 9:30 am, for 3 min, at temperature −110 °C). Blood samples were collected before the first cryostimulation and after completing the last one. Prior to treatment body composition and fitness level were determined. The applied protocol of cryostimulation lead to rise the blood irisin in obese non-active men (338.8 ± 42.2 vs 407.6 ± 118.5 ng mL^{-1}), whereas has no effect in obese active men (371.5 ± 30.0 vs 343.3 ± 47.6 ng mL^{-1}). Values recorded 24 h after the last cryo-session correlated significantly with the fat tissue, yet inversely with the skeletal muscle mass. Therefore, we concluded the subcutaneous fat tissue to be the main source of irisin in response to cold exposures. The applied cold treatment reduced the high sensitivity C-reactive protein (hsCRP) and Hpc concentration confirming its anti-inflammatory effect.

1. Introduction

Obesity is a serious health problem in developed countries, and the prevalence of obesity has increased dramatically for several decades [43]. Wide range of co-morbidities such as: insulin resistance, metabolic syndrome, type 2 diabetes, hypertension, chronic kidney disease, cardiovascular disease, heart failure, cancer, and dementia are associated to obesity through the low-grade inflammation [26]. This stage is characterized by an enhancement of the pro-inflammatory cytokines' concentrations, particularly through their release from adipose cells and macrophages [41]. The elevated pro-inflammatory cytokines might stimulate the synthesis of hepcidin (Hpc), which is a disulfide-rich peptide produced by hepatocytes and an iron-regulating hormone [35]. Hepcidin synthesis is also stimulated by high blood iron concentration [12]. An increased level of blood hepcidin inhibits iron transport from the duodenum, thus limiting iron absorption [33]. The rise of hepcidin concentration found in a chronic state of inflammation can be treated as a non-specific host defense strategy, aimed at limiting the availability of iron for microorganisms [32]. Conversely, in overweight and obese people with a low-grade systemic inflammation, a positive association was found between the C-reactive protein and ferritin [4]. Unexpectedly, obese subjects exhibited low iron concentrations, even anemia, in response to elevated hepcidin concentrations [1].

There are few strategies decreasing chronic low-grade inflammation in obese people. Since low grade inflammation can be associated with physical inactivity in healthy subjects [38], physical

* Corresponding author.
E-mail address: ziemann.ewann@gmail.com (E. Ziemann).

http://dx.doi.org/10.1016/j.cryobiol.2015.10.143
0011-2240/

exercise is one of the mechanisms that may be stimulate the anti-inflammatory response and protection against chronic medical disorders induced by this stage [36]. However, in some cases physical activity was almost completely ineffective in reducing systemic inflammation [25]. Thus, there is a need to look for additional methods supporting the anti-inflammatory strategies. Last papers revealed that the whole-body cryostimulation (CRY) is considered as the an alternative and complementary method to both above mentioned strategies for reducing inflammation [16,29,45]. Nonetheless, still physical fitness level might modify the effect of CRY [45].

Data describing the influence of coldness exposure on irisin is limited [24] with the information about the impact of the whole body cryostimulation on this protein completely lacking. Irisin is a recently discovered myokine, secreted into the circulation following a proteolytic cleavage from its cellular form, fibronectin-type III domain-containing 5 (FNDC5) in response to exercise [6]. Its release is induced during exercise in mice and humans, causing an increase in energy expenditure in mice with no changes in movement or food intake. In addition, irisin activates oxygen consumption in white fat cells, and thermogenesis [22]. Consequently, irisin is considered to be beneficial in the treatment of obesity, diabetes, and a wide range of pathological conditions characterized by an imbalance between energy demand and expenditure [22,40]. Recent papers report that irisin negatively correlates with the BMI, waist-hip ratio, and fat mass in men as well as that circulating irisin is lower in non-diabetic overweight and obese men [17,30,39]. Lee and co-workers noted that cold treatment is one of the factors stimulating irisin secretion. Water-infused thermoblankets (from 27 °C cooled to 18 °C and further lowered by 2 °C every 3 min until 12 °C temperature) was found to have stimulated irisin secretion [24]. Hence, irisin seems to be an important link between cold exposure treatment and obesity.

Overall, basing on the current information about changes stimulated by cryotherapy and its efficiency in reducing low grade systemic inflammation in obese men, the aim of our study was to assess the impact of CRY on irisin concentration among obese men at various fitness level. The second purpose of our investigation was to assess the influence of the whole body cryostimulation on hepcidin concentration and iron status.

2. Materials and methods

2.1. Subjects

Twelve obese men participated in the experiment (38.4 ± 8.2 years of age, height of 179.0 ± 6.0 cm). The participants were categorised as obese based on their BMI according to the current guidelines (BMI >30 kg m^{-2}) and visceral fat area above 100 cm^2 [44]. Additionally, the group was divided into two subgroups according to the participants' cardiorespiratory fitness, which was measured in terms of the maximal oxygen consumption (VO_{2max}). Based on the classification proposed by Astrand [3], subjects with VO_{2max} above 35 mL kg^{-1} min^{-1} were assigned to the high fitness level (HFL) group (weight of 93.8 ± 11.5 kg), whereas those with the lower $VO_{2\ max}$ were enrolled in the low fitness level (LFL) group (weight of 116.5 ± 24.8 kg). All the subjects underwent a medical check-up before being submitted to cold exposure. They were also medication-free. The participants had never previously been subjected to any form of cryotherapy. Written, informed consent was obtained from all subjects. All procedures were approved by the Bioethical Committee of the Regional Medical Society in Gdansk NKEBN/245/2009. One week prior to the start of the experiment, body composition and aerobic capacity were determined for each participant. During the experiment, all participants were instructed not to change any aspect of their daily habits, such as diet, and to avoid any form of exercise.

2.2. Body composition assessment

Body mass (BM) and body composition were estimated using a multi-frequency impedance plethysmograph body composition analyser (In Body 720, Biospace Analyzer, Korea). This analyser accurately measured the amount of body water and body composition, including fat mass, free fat mass, skeletal muscle mass and soft lean mass. Additionally, the visceral fat area was determined and expressed in cm^2. The precision of the repeated measurements was expressed as the coefficient of variation, which was 0.6% for fat mass percentage on average [42].

2.3. Cardiorespiratory fitness measurement

To determine the VO_{2max}, the participants performed a graded cycle ergometry test on an electromagnetically braked, cycle ergometer (ER 900 Jaeger, Germany/Viasys Health Care). The participants were allowed a 5-min warm up period at an intensity of 1.5 W kg^{-1}, with a pedalling cadence of 60 rpm. Immediately following the warm-up, the participants began VO_{2max} testing by cycling at increasingly workloads by 25 W min^{-1} until the participant reached the point of volitional exhaustion [20,45]. The recovery was passive, with the participant in a seated position. Breath-by-breath pulmonary gas exchange was measured (Oxycon-Pro, Jaeger -Viasys Health Care, Germany) throughout the VO_{2max} test; the O_2 and CO_2 analysers were calibrated prior to each test using standard gases of known concentrations in accordance with manufacturer guidelines.

2.4. Whole-body cryostimulation

All of the participants were subjected to a series of coldness exposures (once per day at 9:30 a.m.), including 10 sessions in a cryogenic chamber at the Pomeranian Rheumatologic Centre in Sopot, Poland, which were conducted by highly qualified medical staff. Each cryostimulation session lasted 3 min at a temperature of −110 °C. Entry into the cryo-chamber was preceded by a 20–30 s adaptation period in the vestibule at a temperature of −60 °C. The subjects were dressed in shorts, socks, gloves, and a hat covering their auricles. The examined individuals did not participate in any other treatment after whole-body cryostimulation to avoid obscuring the interpretation of the cryogenic effect. Each exposure was preceded by a light breakfast between 07 and 07:30 a.m. according to the instructions given to the subjects [45].

2.5. Blood analysis and collection

Blood samples were taken from the antecubital vein into the vacutainer tubes with EDTAK $_2$, before, 30 min and 24 h after the first and last cryo-session. Immediately following the blood collection one portion of the sample was transferred to centrifuge tubes containing aprotinin (catalog no RK-APRO) from Phoenix Pharmaceuticals Inc. The final concentration of aprotinin was 0.6 Trypsin Inhibitor Unit/1 mL of blood. The samples were centrifuged at 2000 g for 10 min at 4 °C. The separated plasma samples were frozen and kept at −70 °C until later analysis.

Plasma interleukin IL-6 and high sensitivity C-reactive protein (hsCRP) levels were determined by enzyme immunoassay methods using commercial kits (R&D Systems, USA, catalog no. HS600B, DCRP00 respectively). The average intra-assay CV was 8.0% for IL-6 and hsCRP.

Quantification of plasma irisin was based on a competitive

Please cite this article in press as: K. Dulian, et al., The whole body cryostimulation modifies irisin concentration and reduces inflammation in middle aged, obese men, Cryobiology (2015), http://dx.doi.org/10.1016/j.cryobiol.2015.10.143