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Original research

# High intensity interval exercise enhances the global HDAC activity in PBMC and anti-inflammatory cytokines of overweight-obese subjects

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ABSTRACT

*Objective:* To evaluate the global activity of histone deacetylases (HDACs) in peripheral blood mononuclear cells (PBMC) and systemic levels of cytokines in response to high intensity interval exercise (HIIE) in overweight-obese individuals.
*Methods:* In order to verify the acute effects of exercise on epigenetic and inflammatory modulation, ten overweight-obese males (BMI >25 to <35 kg/m$^2$) performed a single bout of HIIE (10 bouts of 60 s to 85 −90%$P_{Max}$/75 s to 50%$P_{Max}$) and the blood samples were collected pre, immediately post and 24 h post HIIE session for cytokine measurements and HDAC activity analyses. The global activity of HDAC in PBMC was determined by fluorometric assay and the serum concentrations of IL-6, IL-10, IL-17a, TGF-β and TNF-α were quantified by ELISA.
*Results:* HIIE session induced a leukocytosis (27%, $p = 0.001$), characterized by the increase in the number of circulating lymphocytes (41%, $p = 0.002$) and monocytes (59%, $p = 0.001$). Interestingly, a significant increase in global HDAC activity in PBMC (132%, $p = 0.002$) with a concomitant increase in serum concentrations of IL-6 (11%, $p = 0.04$), IL-10 (27%, $p = 0.003$) and TGF-β (63.44%, $p = 0.05$) were observed immediately after exercise. At this time, no significant difference was observed in the levels of TNF-α and IL-17a. None of these variables was significantly changed at 24 h after HIIE.
*Conclusion:* These data support the hypothesis that the anti-inflammatory effects of exercise may be related, at least in part, to the modulation of HDAC activity in PBMC of individuals with overweight-obese.



## 1. Introduction

In 2014, according to the World Health Organization, more than 1.9 billion adults were overweight; wherein of these over 600 million were obese (WHO, 2012). Chronic low-grade inflammation, characterized by 2–4 fold elevation in pro-inflammatory cytokine levels, is found in overweight-obese, sedentary and individuals suffering from cardiometabolic diseases (O'Rourke et al., 2006). It is correlated with hypertrophy of adipocytes, infiltration and phenotype changes of immune cells into adipose tissue, and increased expression pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), with reduction of anti-inflammatory cytokines (O'Rourke et al., 2006; Schipper et al., 2012). In addition, obese presents a pivotal imbalance between regulatory T cells (Treg) and pro-inflammatory Th1 and Th17 cells (Wagner et al., 2013; Winer et al., 2009).

Several authors have reported the importance of epigenetic mechanisms, in particular acetylation and deacetylation of histones, in gene expression under physiological and pathological

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conditions, exhibiting a pivotal role in inflammation and immune response (Suárez-Álvarez et al., 2013; Obata et al., 2015). Histone acetylation is regulated by histone acetyltransferases (HATs), which add acetyl groups to the amino-terminal tails of histones, and histone deacetylases (HDAC), which remove these acetyl groups, events widely associated with enhanced transcriptional activity and transcriptional repression, respectively (Suárez-Álvarez et al., 2013). Moreover, it was demonstrated that HDACs regulate the acetylation of a great number of non-histone proteins, including GATA-1, GATA-3, STAT-3, STAT-5, Foxp3, NF-kB, which are critical for many immunological events (Sweet et al., 2012). In addition, many sub-types of HDAC are able to induce tolerogenic patterns thought increased synthesis of anti-inflammatory and immunomodulatory cytokines, thereby HDAC may be a potential target in the treatment of obesity (Obata et al., 2015; Abu-Farha et al., 2013).

The current exercise guidelines recommend an amount of at least 150 min per week for the treatment and prevention of several diseases (Gleeson et al., 2011; Jensen et al., 2014). However, most people do not perform regular physical activities due to lack of time. In this sense, the high intensity interval exercise (HIIE) emerges as an alternative because of its characteristic of being time-efficient, i.e. lead to cardiorespiratory and metabolic adaptations similar to traditional exercises, but with low volume per session (Gillen and Gibala, 2014).

Considering that HIIE provides physiological adaptations similarly to conventional aerobic exercise, it is reasonable to assume that it can induce immunological adaptations with anti-inflammatory effects. There are few studies in literature demonstrating its benefic effects in obese subjects (Dorneles et al., 2016; Leggate et al., 2012). Horsburgh et al. (2015) pointed that epigenetic modifications, such as DNA methylation and post-translational histone modifications, may explain the immunoregulatory effects of physical exercise. However, there are few studies focusing the epigenetic changes on immune cells after an acute bout of exercise. Recently, Robson-Ansley et al. (2014) demonstrated that global methylation and CpG site-specific methylation remained unchanged after acute strenuous exercise (120 min of treadmill running at 60%v$VO_{2Max}$ followed by 5 km time trial), despite the fact of IL-6 levels correlated with CpG methylation at 11 CpG sites. As far as we know, there is no data showing the influence of physical exercise on histone deacetylase activity in leukocytes, and its relationship with anti-inflammatory effects. Thus, the aim of this study was to evaluate the effect of one session of HIIE on the global activity of HDAC in peripheral blood mononuclear cells (PBMCs) and systemic inflammatory markers in overweight-obese individuals.

## 2. Methods

### 2.1. Subjects

Ten sedentary overweight-obese men participated in this study. Overweight-obesity grade 1 was defined as body mass index (BMI) greater than 25 kg/$m^2$ and lower than 35 kg/$m^2$ according to the criteria of World Health Organization (WHO, 2012). This study was approved by the Ethic Committee of Centro Universitário Metodista IPA (Porto Alegre, Brazil) (626.668) and all experimental procedures were performed according to the Declaration of Helsinki. All the subjects were informed about the study and signed the informed consent. They completed a medical questionnaire and the Physical Activity Readiness Questionnaire previous to experimental trial.

Participants were normo-glycemic and free of illness and injury and they were not engaged in any physical training programs for a period of six months prior to the experimental trials. None of the participants were smokers or in use of glucocorticoids for at least four weeks before the data collection and should refrain from alcohol or caffeinated drinks in 48 h priors to 24 h after the exercise session. The control of these variables was done due to the fact that some corticosteroids can affect the activity of the HDAC and the inflammatory response, and because tabagism and caffeine can modulate the immune response (Horrigan et al., 2006; Ito and Adcock, 2002).

### 2.2. Preliminary measurements

One week before the main trial, the subjects completed a maximal exercise test in a treadmill ergometer and a single session of protocol familiarization; each step was performed with an interval of at least three days.

### 2.3. Testing procedure

All participants completed an incremental exercise test to volitional exhaustion in a treadmill ergometer (Inbramed Millenium ATL, Inbrasport, Porto Alegre, Brazil) in the Exercise Physiology Laboratory of Centro Universitário Metodista IPA (Porto Alegre, Brazil). During the test, temperature and humidity were continuously measured (temperature ranged from 18 to 24 °C and humidity from 50 to 70%). After a brief warm-up of 5 km/h during three minutes, the speed was gradually increased by 1 km/h each minute until exhaustion. The treadmill inclination was set at 1% to simulate best running outdoor conditions. Heart rate was monitored continuously during the test and HIIE session with a heart rate monitor (Polar Electro FT7, Kempele, Finland), and the perceived exertion was recorded according to the Borg Scale (Borg, 1974). . When the participants reached the following criteria: (a) heart rate ≥predicted for age; (b) perceived exertion >18; or (c) when participant voluntarily stopped, the test was terminated. The participants were verbally encouraged to run until the exhaustion. After the test, the speed associated with $VO_{2Peak}$ ($S_{Max}$), the maximal power output ($P_{Max}$), the maximal heart rate ($HR_{Max}$) and the perceived exertion maximal were recorded (Dorneles et al., 2016). The $S_{Max}$ was used for determine the individual HIIE session.

### 2.4. Familiarization session

Participants returned to the laboratory three days after the progressive test to the familiarization session. After five minutes of warm-up (70%$P_{Max}$), individuals performed five bouts with duration of 60 s (90%$P_{Max}$) with 60 s of active rest (50%$P_{Max}$).

### 2.5. High intensity interval exercise session

The subjects arrived at the laboratory between 9:30 and 10:30 a.m. in fasting for at least one hour. To perform the high intensity interval exercise (HIIE), we adapted the protocol suggested by Gillen and Gibala (2014). The session consisted of 10 × 60 s bouts interspersed with 75 s of recovery. Exercise was performed on a treadmill ergometer (Inbramed Millenium ATL, Inbrasport, Porto Alegre, Brazil). Individual workloads were selected to elicit an 85–90% of $S_{Max}$ during the intervals. During the recovery, the intensity was adjusted to 50% of $S_{Max}$. A five minutes cool-down at 50% of $S_{Max}$ was included at the end of HIIE session, and the total session time was 31 min. Heart rate (HR) and perceived exertion (RPE) were recorded at the end of each interval. After the exercise session, participants remained seated to collect 10 mL of venous blood under conditions identical to the pre-session collection; the same procedure was performed 24 h after the session.