Journal of Proteomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Proteomics

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

Can half-marathon affect overall health? The yin-yang of sport

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ARTICLE INFO

Keywords:
RUNX2
PPARG
Half marathon
Plasma proteomics

ABSTRACT

Physical activity improves overall health and counteracts metabolic pathologies. Adipose tissue and bone are important key targets of exercise; the prevalence of diseases associated with suboptimal physical activity levels has increased in recent times as a result of lifestyle changes. Mesenchymal stem cells (MSCs) differentiation in either osteogenic or adipogenic lineage is regulated by many factors. Particularly, the expression of master genes such as RUNX2 and PPARγ2 is essential for MSC commitment to osteogenic or adipogenic differentiation, respectively. Besides various positive effects on health, some authors have reported stressful outcomes as a consequence of endurance in physical activity. We looked for further clues about MSCs differentiation and serum proteins modulation studying the effects of half marathon in runners by means of gene expression analyses and a proteomic approach. Our results demonstrated an increase in osteogenic commitment and a reduction in adipogenic commitment of MSCs. In addition, for the first time we have analyzed the proteomic profile changes in runners after half-marathon activity in order to survey the related systemic adjustments. The shotgun proteomic approach, performed through the immuno-depletion of the 14 most abundant serum proteins, allowed the identification of 23 modulated proteins after the half marathon. Interestingly, proteomic data showed the activation of both inflammatory response and detoxification process. Moreover, the involvement of pathways associated to immune response, lipid transport and coagulation, was elicited. Notably, positive and negative effects may be strictly linked. Data are available via ProteomeXchange with identifier PXD006704.

Significance: We describe gene expression and proteomic studies aiming to an in-depth understanding of half-marathon effects on bone and adipogenic differentiation as well as biological phenomena involved in sport activity.

We believe that this novel approach suggests the physical effects on overall health and show the different pathways involved during half marathon.

Contents of the paper have not been published or submitted for publication elsewhere. The authors declare no conflict of interest.

1. Introduction

The half-marathon (HM, 21.1 km) is one of the most popular outdoor recreational activities, attracting an increasing number of participants around the globe. The basic reasons for its popularity entail a lower physiological demand compared to longer distance contests, such as marathon and ultra-marathon, combined with the renowned benefits for health and fitness that are typically associated with endurance exercise [1]. Endurance running has been the basis of human activities over an extended period of time, determining the natural selection of the metabolic profiles specifically addressed at satisfying the energetic requirements [2]. Only very recently, in evolutionary terms, mankind has turned to a sedentary lifestyle so the energy balance has become positive. This has led to the exponentially increased prevalence of several diseases whose pathogenesis is associated with suboptimal physical activity levels [3]. Physical inactivity has been recognized, by

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http://dx.doi.org/10.1016/j.jprot.2017.09.004
Received 22 June 2017; Received in revised form 18 August 2017; Accepted 5 September 2017

the World Health Organization (WHO) as the fourth death cause (5.5% of deaths globally) following hypertension (12.8%), smoking (8.7%), and hyperglycaemia (5.8%), and preceding obesity and overweight (4.8%) [4]. It is well established that an important key target of exercise is the adipose tissue. Regular exercise decreases the size of adipocytes and increases their insulin sensitivity [5,6]. Through the secretion of adipokines, adipocytes exert endocrine and autocrine functions aimed at modifying insulin sensitivity at the liver and skeletal muscle level, as well as in the adipose tissue itself. By modulating adipokines expression, physical activity is a powerful lipolytic stimulus in the visceral adipose tissue, contributing also to reduce the central adiposity, thus improving systemic inflammation [7]. Endurance running, among the few modifiable factors, is also one of the main determinants of bone mass and bone metabolism. In particular, it is an acknowledged source of bone turnover and is recommended for preventing osteoporosis and bone metabolism problems [8]. Cross-sectional studies on BMD and other bone strength markers have shown runners to be advantaged when compared to inactive controls [9].

Recently, it has been reported that Mountain Ultra-Marathon (MUM) increases bone formation rate in runners. In fact, in ultra-marathon runners, levels of PINP, a serum procollagen I N-terminal propeptide associated to bone formation, were enhanced; they were higher in MUM participants than in moderately trained individuals (control group) [10]. While it is known that a restrained physical activity improves health and promotes wellbeing, a stressful physical activity may trigger adverse effects [11,12]. However, even if previous studies among runners have shown that muscle, heart as well as kidney injury may occur [13–15], data related to systemic interactions are lacking. Interestingly, in a recent study it has been shown that either marathon or half-marathon activity can affect some inflammation mediators and, consequently, can induce immune system reactivity in runners [14]. A limit of the above study was the small sample size: marathon (n = 4), half-marathon (n = 4); these findings nevertheless suggested a systemic involvement in response to physical activity.

It has been reported that while a chronic endurance training reduces telomere shortening thus counteracting the aging process, oxidative stress due to marathon participation affects telomere length due to DNA damage [16]. This finding suggests that a single stressful activity can induce specific molecular pathways which are also activated in some diseases. Consequently, additional information related to the effects of intense physical activity is required in order to be aware of and possibly counteract adverse consequences.

Though in the last years many studies have investigated the effects of stressful physical activity in runners [14,16], the effects of half-marathon on osteogenic and adipogenic differentiation as well as the variations in proteomic profiles are still lacking. The aim of the present study was to investigate the consequences of half-marathon on osteogenic and adipogenic differentiation in order to evaluate the effects of this physical activity on skeletal and adipose tissue. In addition, for the first time we have analyzed the proteomic profile changes in runners after half-marathon activity in order to survey the related systemic adjustments.

2. Materials and methods

2.1. Subjects and sera collection

The study was conducted during a sport event called ‘Run For Science’, held in Verona (Italy) in April 2016, which was specifically planned to investigate the effects of distance running on recreational athletes. Eleven amateur runners (median age 41,4 ± 10,1) carried out a 21.1 Km half marathon. Blood samples, obtained by venipuncture, were collected before the run and immediately after. All participants gave informed consent. Sera, obtained frome collected before the run and immediately after. All participants gave informed consent. Sera, obtained from 10 mL of fresh blood by centrifugation at 400 × g., were harvested and frozen in aliquots at − 80 °C until use.

2.2. Osteogenic and adipogenic differentiation of mesenchymal stem cells

We used hMSCs (PromoCell) to analyze the effects of sera obtained from runners before (pre-sera) and after (post-sera) the competition on adipogenic and osteogenic differentiation. We chose commercial MSCs in order to avoid confounding effects of different circulating growth factors as well as cytokines. Sera pools (pre- and post-run, respectively) were obtained mixing equal serum volumes from all participants. Serum was added to medium at 10% concentration. Cells were then plated at a density of 5×10^4 cells per well into 48-well plates and cultured for 1 week before gene expression analysis.

2.3. Total RNA extraction

Total RNA was obtained by RNAeasy minikit (Quiagen) with DNAse I treatment. The extracted RNA was quantified by measuring the absorbance at 260 nm and the purity checked by measuring the 260/280 absorbance ratio.

2.4. Reverse transcription

First-strand cDNA synthesis was performed with the First Strand cDNA Synthesis Kit (GE Healthcare), by using random hexamers, (GE Healthcare) and according to the manufacturer's protocol. The product was then aliquoted in equal volumes and stored at − 80 °C.

2.5. Real time RT-PCR

PCRs were performed in a total volume of 25 μL containing 1 × Taqman Universal PCR Master mix, no AmpErase UNG and 2.5 μL of cDNA from each sample; pre-designed, Gene-specific primers and probe sets for each gene (*Runx2*, Hs00231692_m1; *PPARΓ2*, Hs01115513_m1; B2M, Hs999999_m1; GAPDH, 0802021; Applied Biosystems) were obtained from Assay-on-Demande Gene Expression Products (Applied Biosystems). Real Time PCR reactions were carried out in multiplex. RT amplifications included 10 min at 95 °C (AmpliTaq Gold activation), followed by 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. Thermocycling and signal detection were performed with ABI Prism 7300 Sequence Detector (Applied Biosystems). Ct values for each reaction were determined using TaqMan SDS analysis software. For each amount of RNA tested triplicate Ct values were averaged. Since Ct values vary linearly with the logarithm of the amount of RNA, this average represents a geometric mean. In addition, we considered a fold change < 0.8 and > 1.2 to be relevant, even if smaller changes were statistically significant.

2.6. Alizarin red staining

In order to evaluate osteoblastic maturation, cells were cultured for 28 days with pre- and post-sera, subsequently. Fixed with 70% ethanol and rinsed with deionized water. Then, cells were treated for 5 min with 40 mM Alizarin red S at pH 4.1, and gently washed with 1 × phosphate-buffered saline for 15 min.

2.7. Proteomic analysis

2.7.1. Sera sample preparation

Twelve microliters of sera were depleted of high abundant proteins using the Seppro IgY14 spin column kit (Sigma-Aldrich Inc., St. Louis, MO, USA) following the manufacturer protocol. The method is used to bind human serum HSA, IgG, fibrinogen, transferrin, IgA, IgM, haptoglobin, alpha2-macroglobulin, alpha1-acid glycoprotein, alpha1-antitrypsin, Apo A-I HDL, Apo A-II HDL, complement C3 and LDL (ApoB) and thus to increase the identification of low-abundant proteins. The