Free Radical Biology and Medicine 98 (2016) 46–55

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Exercise-induced ROS in heat shock proteins response

Ivan Dimauro [1], Neri Mercatelli [1], Daniela Caporossi *

Unit of Biology, Genetics and Biochemistry, Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Piazza Lauro De Bosis 15, 00135 Rome, Italy

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form
18 March 2016
Accepted 24 March 2016
Available online 26 March 2016

Keywords:
Heat shock proteins
Exercise
Reactive oxygen species
Redox signaling

ABSTRACT

Cells have evolved multiple and sophisticated stress response mechanisms aiming to prevent macromolecular (including proteins, lipids, and nucleic acids) damage and to maintain or re-establish cellular homeostasis. Heat shock proteins (HSPs) are among the most highly conserved, ubiquitous, and abundant proteins in all organisms. Originally discovered more than 50 years ago through heat shock stress, they display multiple, remarkable roles inside and outside cells under a variety of stresses, including also oxidative stress and radiation, recognizing unfolded or misfolded proteins and facilitating their restructuring. Exercise consists in a combination of physiological stresses, such as metabolic disturbances, changes in circulating levels of hormones, increased temperature, induction of mild to severe inflammatory state, increased production of reactive oxygen and nitrogen species (ROS and RNS). As a consequence, exercise is one of the main stimuli associated with a robust increase in different HSPs in several tissues, which appears to be also fundamental in facilitating the cellular remodeling processes related to the training regime. Among all factors involved in the exercise-related modulation of HSPs level, the ROS production in the contracting muscle or in other tissues represents one of the most attracting, but still under discussion, mechanism. Following exhaustive or damaging muscle exercise, major oxidative damage to proteins and lipids is likely involved in HSP expression, together with mechanically induced damage to muscle proteins and the inflammatory response occurring several days into the recovery period. Instead, the transient and reversible oxidation of proteins by physiological concentrations of ROS seems to be involved in the activation of stress response following non-damaging muscle exercise. This review aims to provide a critical update on the role of HSPs response in exercise-induced adaptation or damage in humans, focusing on experimental results where the link between redox homeostasis and HSPs expression by exercise has been addressed. Further, with the support of *in vivo* and *in vitro* studies, we discuss the putative molecular mechanisms underlying the ROS-mediated modulation of HSP expression and/or activity during exercise.

1. Introduction

Cellular stress response represents an adaptive protective mechanism to maintain or re-establish cellular homeostasis so to survive under unfavorable environmental conditions, such as heat shock, hypoxia and oxidative damage [1]. The mechanisms of stress response commonly include a transient down-regulation of growth related proteins, whereas the production of specific stress-response proteins increases. Strictly dependent on type, intensity and duration, the stressing stimulus can promote either cell survival with adaptation to adverse conditions or the elimination of excessively damaged cells [2].

Heat shock proteins (HSPs) are the most highly conserved stress response proteins during evolutionary history [1]. In humans, they display different functions depending on tissue specific localization, intra- or extracellular distribution, developmental expression, level of induction and the client proteins they interact with (Table 1). As common mechanism of action, HSPs recognize unfolded or misfolded proteins and facilitate their restructuring in either an ATP-dependent (large HSPs) or energy independent manner (low weight HSPs) [3]. Indeed, HSPs not only protect the cells against proteotoxic stresses but they have also a critical role in normal functioning of several cellular processes, such as the assembly of multiprotein complexes and the transport of proteins across cellular membranes [4,5]. Moreover, HSPs are fundamental for the maintenance of cell structural integrity, interacting with cytoskeletal elements, influencing their formation and function, and protecting them during proteotoxic stress [6]. Under several stress conditions, HSPs are highly up regulated by heat shock factors (HSFs) in order to maintain cellular homeostasis and to enhance cell survival functions [7].

* Corresponding author.
E-mail address: daniela.caporossi@uniroma4.it (D. Caporossi).
[1] These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.freeradbiomed.2016.03.028
0891-5849/

Table 1
The main heat shock proteins and their intracellular localization. Both weight-based and HGNC (in branched) nomenclatures are shown.

Heat shock protein	Localization
Hsp110 *(HSPH2)*	Cytosol/nucleus
Hsp90 *(HSPC1)*	Cytosol
Hsp70/Hsp72* *(HSPA1A)*	Cytosol/nucleus
Hsc70 *(HSPA8)*	Cytosl/nucleus
Hsp60 *(HSPD1)*	Mitochondria
αA crystallin *(HSPB4)*	Cytosol/nucleus
αB crystallin *(HSPB5)*	Cytosol/nucleus
Hsp25/Hsp27 *(HSPB1)*	Cytosol/nucleus
Hsp22 *(HSPB8)*	Mitochondria
Hsp20 *(HSPB6)*	Cytosol/nucleus

* always referred as Hsp70 throughout the text

The most characterized HSPs families include the Hsp90 (HSPC) family, with five well-characterized members, the Hsp70 (HspA) group with 13 members, the Hsp60 (HSPD) family with only one form, mostly found in the mitochondrial matrix, and the family of the small HSPs (sHSPs), which includes 11 members, among which are the well-studied members Hsp27 (HSPB1) and αB crystallin (HSPB5) [8].

Voluntary muscle contraction is associated with a production of several stressors generated for example by metabolic disturbances, changes in circulating levels of hormones, increased temperature, induction of mild to severe inflammatory state, increased production of reactive oxygen and nitrogen species (ROS and RNS), and other free radicals [9,10]. Skeletal muscle and other tissues distinguish the different signals which are specific to the nature, intensity and duration of exercise, so that the categorization of exercise stressors and exercise-induced stress response can vary greatly depending upon which type of exercise protocol has been utilized [11]. During acute exercise, the type and the magnitude of stress response depend on the short-term modification of cellular homeostasis and on the repair of damage eventually inflicted to cellular components [12]. Conversely, in exercise training programs, with repetitive exercise administered in a “chronic” form, the stress response plays a role in the accumulative physiological adaptation that maintains homeostatic balance, and it represents a crucial component of the cellular and molecular mechanisms by which regular exercise confers protection against related and unrelated stressors [13]. Since exercise consists in a combination of physiological stresses, it is not surprising that it acts as a potent HSPs inducer. Their increase following voluntary muscle contraction in muscle and other tissues is strictly related to the type and magnitude of exercise stressors [14]. For instance, while non-muscle damaging isotonic contractions involve mostly the modulation of Hsp70 and Hsp60, eccentric contractions, considered as a muscle damaging exercise, involve also the phosphorylation and translocation of small HSPs [15].

Among all factors involved in the exercise-related modulation of HSPs level, the ROS production in the contracting muscle or in other tissues represents one of the most attracting, but still under discussion, mechanism [16]. The majority of reports have assumed that, during exercise, the skeletal muscle provides the main source of ROS and RNS. These molecules play an important role, not only in the skeletal muscle adaptation to exercise, but also in the muscle plasticity that occurs during a period of prolonged inactivity. Low/moderate amounts of ROS produced during regular muscle contraction are considered to evoke specific adaptations, such as an increased activity of antioxidant and/or oxidative damage repair enzymes, increased resistance to oxidative stress and lower levels of oxidative damage [17]. ROS function as messenger in exercise-induced adaptive gene expression and exercise can be considered a potent hormetic conditioner [18].

The variations in exercise type and protocol utilized in different studies, the differences in subject characteristics within and between studies [10], as well as the difficulty of the direct measurement of ROS production in living systems [19], pose clear limitations to our understanding of the net contribution of exercise-induced ROS in the modulation of HSP response. Nevertheless, the transient and reversible oxidation of proteins by physiological concentrations of ROS seems to be involved in the activation of stress response following non-damaging endurance-type of exercise, while mechanical disruption of protein structure and secondary inflammatory processes are likely involved in the stress response following forms of “damaging” exercise [10].

In the following sections of this review, we provide a critical update on the role of HSPs response in exercise-induced adaptation or damage in humans, then focusing on experimental results where the link between redox homeostasis and HSPs expression by exercise has been addressed. Further, with the support of *in vivo* and *in vitro* studies, we discuss on the putative molecular mechanisms underlying the ROS-mediated modulation of HSP expression and/or activity during exercise.

1.1. HSPs response in the cellular adaptation to exercise

It is widely accepted that acute or chronic exercise modulates the activity and the expression of HSPs in several human tissues such as the skeletal muscle, as well as in circulating monocytes and lymphocytes, or body fluids [20–22]. Moreover, results from animal studies clearly indicate that any forms of exercise induce changes in the level of HSPs in spleen, heart, liver, kidney, placenta, brain and spinal cord [23–27]. As already indicated, the magnitude of the exercise stress plays a major role in the stress response [14], while with a repetitive exercise, the initial response of some HSPs can be lower as training progresses [10]. The exercise induced changes in HSPs seem to have multiple cytoprotective effects on mitochondria and on sarcoplasmic reticulum and cytoskeleton components [28–30], inhibitory effects on apoptosis [31], as well as a role in the maintenance of enzymatic activity, insulin sensitivity and glucose transport [32,33].

Most of the human studies from endurance aerobic and resistance anaerobic exercises refer to the modulation of Hsp70 in skeletal muscle and/or in circulating monocytes [20,34–37]. Hsp70 is the most abundant of all HSPs [38], accounting for 1–2% of cellular protein being highly represented in skeletal muscle. It is known that a single bout of exercise is sufficient to increase Hsp70 in skeletal muscle at both mRNA and protein levels in an intensity dependent manner [14]. Differently from other HSPs, baseline levels of Hsp70 are increased by prolonged exercise training [39,40], while mechanical unloading determines a decrease in the Hsp70 content in muscle [41]. Recently, Cobley et al. [42] demonstrated that aging was associated with a lower Hsp70 increase post acute exercise in the *vastus lateralis* of untrained and trained old subjects, although the increase in Hsp70 was greater in trained compared with untrained individuals.

As molecular chaperones, Hsp70 helps the correct refolding of nascent proteins and interacts with unfolded proteins to avoid inappropriate interactions and degradation of damaged proteins [43]. In addition, it has a role in protein translocation, anti-inflammatory responses, control of cell signaling, modulation of immune response and chronic disease conditions, such as diabetes, obesity, and insulin resistance [44]. Indeed, a growing number of evidences from animal studies verify the relevance of exercise-induced Hsp70 in the maintenance of cellular functions and disease prevention. Smuder et al. [45] show that the increase