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Heat-induced extracellular HSP72 release is blunted in elderly diabetic people compared with healthy middle-aged and older adults, but it is partially restored by resistance training

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ABSTRACT

Recent evidence suggests that the anti-inflammatory heat shock response (HSR) is reduced in aging and diabetes. In this study we compared HSR between healthy middle-aged adults, healthy elderly and type 2 diabetic (T2DM) elderly, and tested whether resistance training (RT) could improve the HSR in T2DM group. Thirty sedentary participants volunteered for this study. HSR (assessed as the capacity to export HSP72 during heat stress) was measured in the blood and compared between the groups. HSR was similar between healthy middle-aged and healthy elderly volunteers, but diminished in elderly T2DM ($p < 0.001$). Hence, T2DM subjects ($n = 12$) were submitted to a 12-week RT program, because exercise is a physiological HSR inducer. HSR, cytokines, metabolic parameters and visceral adipose tissue (VAT) were measured before and after the RT. Remarkably, VAT was negatively correlated with HSR ($r = -0.49$, $p < 0.01$) while RT improved the HSR and reduced inflammation [TNF-α: from 51.5 ± 9 to 40.7 ± 4 pg/mL and TNF-α/IL-10 ratio: from 1.55 ± 0.3 to 1.16 ± 0.2 ($p < 0.001$)], without affecting other parameters. All together, these findings confirm the hypothesis that the anti-inflammatory HSR is depressed in elderly diabetic people, but can be partially restored by RT.

1. Introduction

Aging is a natural process that is associated with declines in different physiological systems, including structural and functional deteriorations to the cardiovascular system and unfavourable changes in body composition, such as increased visceral adipose tissue (VAT) and declines in skeletal muscle mass and strength (Jaqueline Santos Moreira Leite et al., 2016). Although these changes are expected, inadequate diet and reduced levels of physical activity can accelerate these aging-related dysfunctions. Another intriguing characteristic of human aging is the reduced ability to maintain cellular homeostasis in the face of adverse environmental stresses (Rao et al., 1999). For this reason,

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elderly people may become more susceptible to different types of diseases of inflammatory nature.

The heat shock response (HSR) is a highly conserved transcriptional program that is mandatory for all cells to efficiently respond to a wide range of stressors, such as oxidative stress, thermal stress, ischemia, exercise, metabolic challenges and many others (Krause et al., 2015a). In addition, HSR is a key component of the physiological response to resolve inflammation (Newsholme and de Bittencourt, 2014). The activation of HSR leads to the increased expression of a group of proteins named as heat shock proteins (HSPs). Among them, the 72 kDa member of the 70 kDa family of heat shock proteins, HSP70 (encoded by the *HSPA1A* gene in humans), is the most abundant of all HSPs, accounting for 1–2% of cellular protein, and is plentiful in many tissues, including the skeletal muscle (Noble et al., 2008).

The functions of the HSP72 include protecting thermally damaged proteins from aggregation, unfolding aggregated proteins, refolding damaged proteins or targeting them for efficient degradation (thus, acting as a molecular chaperone) (Madden et al., 2008). Other functions include protein translocation, anti-apoptotic, anti-inflammatory and, more recently, the HSP roles have been expanded to incorporate the control of cell signalling and immune responses (Krause et al., 2015a), as well. This protein is known to be expressed and released into the extracellular space having a wide variety of effects on other cells (Tytell, 2005). For instance, HSP72 affects and modulates the physiology of immune cells (activation/inactivation), and it is involved in the inducement of neural cell protection under stress conditions (Krause and Rodrigues-Krause Jda, 2011). Interestingly, HSP72 can induce antagonistic actions, depending on its location. For example, intracellularly (iHSP72)exerts a powerful anti-inflammatory effect, while extracellularly (eHSP72) may activate pro-inflammatory pathways (Krause et al., 2014). Increased eHSP70 is associated with inflammatory and oxidative stress conditions, whereas decreased iHSP70 levels are related to insulin resistance in the skeletal muscle (Henstridge et al., 2014). In addition, it is possible that eHSP72 may also contribute to metabolic homeostasis by actively restoring HSP72 content in insulin resistant tissues containing low endogenous levels of HSPs (Archer et al., 2017).

In healthy cells, the HSR encompasses the ability, when under stress, to increase the expression of iHSP72 and its further exportation as eHSP72, by cells that can systematically release this protein (such as blood mononuclear cells). Since this response is essential for proteostasis and resolution of inflammation, a hampered HSR may lead to several chronic inflammatory-related diseases, such as Alzheimer, insulin resistance, type 2 diabetes mellitus (T2DM) and other age-related chronic-degenerative diseases (Krause et al., 2015a; Archer et al., 2017; Hooper et al., 2014). Hence, the maintenance of a normal HSR is crucial to avoid these conditions and, for this reason, interventions such as the heat therapy (Krause et al., 2015b; Faulkner et al., 2017) and exercise training (Krause et al., 2015a), known inducers of the HSR, can be used as non-pharmacological tools in order to improve health. However, it seems that there is no study so far focusing on the evolution of the HSR trough the life span, and its possible associations with T2DM.

Therefore, the aim of this study was to compare the HSR between healthy middle-aged adults (45–59 years old), healthy elderly adults (> 60 y.o.) and T2DM elderly people (> 60 y.o.), in order to understand the changes of this response during aging and diabetes. In addition, considering the known positive effects of resistance training exercise on metabolic responses in T2DM, we also aimed to test the effects of resistance training (RT) over the HSR, plasma cytokine levels, metabolic parameters and VAT, in diabetic elderly people.

2. Methods and materials

2.1. Participants characteristics and ethics

Thirty (19 females and 11 males) sedentary non-smoking participants volunteered for this study (11 healthy middle-aged adults, 7 healthy old adults and 12 diabetic old subjects, previously diagnosed by their personal physicians). Body mass index (BMI) ranged from 22 to 29.8 kg/m^2 for healthy subjects and 24 kg/m^2 to 34.9 kg/m^2 for T2DM people. Informed consent form was obtained from all participants prior to the beginning of the study. Research assessments and protocols were approved by the local Ethics Committee. All healthy subjects were reported as non-diabetic after interview with an experienced physician who considered their fasting glycaemia as the main criteria for diabetes exclusion. Inclusion criteria for diabetic people were $HbA_{1c} \geq 6.5\%$ (within the last six months), controlled blood pressure, and no use of insulin. People were classified as middle-aged (between 45 and 59 years old) or old adults (> 60 y.o.). Participants were excluded if they reported a history of myocardial infarction, cardiac illness, vascular disease, stroke, major systemic disease or any condition that would prevent them from engaging in an exercise study; or if they had already been engaged in two or more planned and structured exercise sessions per week (in the last six months). In addition, the cardiovascular condition was verified by an experienced cardiologist and included also a stress electrocardiogram (ECG) test. The study procedures were conducted according to the Declaration of Helsinki, and all participants provided written informed consent. This study is registered at ClinicalTrials.gov (NCT03489083).

2.2. Anthropometric and visceral adipose tissue (VAT) measurements

Standing height was measured using a Stainless Steel Stadiometer (Urano, Canoas, Brazil), with the participants' shoes off and head at the Frankfort horizontal plane. Body mass was measured using a weighing scale (Urano, Canoas, Brazil). VAT was assessed using ultrasonography (Nemio XG ultrasound, Toshiba, Japan). All VAT measurements were performed with the subjects fasting, in supine position, and visceral fat was measured at the end of a normal expiration with the vertebral column positioned horizontally. VAT ultrasound measurements were performed with a 38-mm, 3,75 -MHz convex-array probe that was placed directly above the umbilical scar. VAT thickness was considered as the distance between the posterior part of the rectus abdominal muscle and the posterior wall of abdominal artery (Sankar et al., 2012).

2.3. Blood sampling and general biochemistry

Venous blood samples were taken after fasting from an antecubital vein in heparin coated and gel-clot Vacutainer™ tubes using standard aseptic techniques. Samples were immediately centrifuged (at 4 °C and 1000 × *g* for 15 min), after which plasma and serum was removed and stored at −80 °C for further analysis. Plasma lipid profile, glycaemia and high-sensitive C-reactive protein (hsCRP) levels were measured in an automated system Cobas C111 (Roche Diagnostics, Basel, Switzerland). Data were expressed as mg/dL for CRP and as % of total haemoglobin for HbA_{1c}.

2.4. Heat shock response test

Considering the importance of the HSR for stress adaptation, we tested the capacity of leukocytes (a major source of circulating HSP72 and representative of immune cell stress response), to release HSP72, under heat stress conditions (a normal and expected response in healthy cells). We used this strategy to compare the HSR in different populations and to test if our intervention could improve the HSR in diabetic elderly people, by testing before and after the resistance training (RT). Briefly, after harvesting, whole blood was immediately incubated at two different temperatures: 37 °C (control) and 42 °C (heat stressed) for 2 h in a water bath. After incubation, total blood was centrifuged to isolate plasma. Plasma was used for the analysis of eHSP72.