Annals of Anatomy 200 (2015) 44–53

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Research article

Morphometric analysis of somatotropic cells of the adenohypophysis and muscle fibers of the psoas muscle in the process of aging in humans

Vladimir M. Antić[a,*], Natalija Stefanović[a], Ivan Jovanović[b], Milorad Antić[c], Miroslav Milić[d], Miljan Krstić[e], Braca Kundalić[b], Verica Milošević[f]

[a] University of Niš, Faculty of Sport and Physical Education, Čarnojevića 10a, 18000 Niš, Serbia
[b] University of Niš, Medical Faculty, Department of Anatomy, Bul. Dr Zoran Đinđić 81, 18000 Niš, Serbia
[c] University of Niš, Medical Faculty, Bul. Dr Zoran Đinđić 81, 18000 Niš, Serbia
[d] University of Niš, Medical Faculty, Department of Forensic Medicine, Bul. Dr Zoran Đinđić 81, 18000 Niš, Serbia
[e] University of Niš, Medical Faculty, Department of Pathological Anatomy, Bul. Dr Zoran Đinđić 81, 18000 Niš, Serbia
[f] University of Belgrade, Institute for Biochemical Research "Siniša Stanković", Bul. Despota Stefana 142, 11000 Beograd, Serbia

ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 23 January 2015
Accepted 28 January 2015

Keywords:
Sarcopenia
Type 1 muscle fibers
Type 2 muscle fibers
Somatotropes
Morphometry

ABSTRACT

The aim of this research was to quantify changes of the adenohypophyseal somatotropes and types 1 and 2 muscle fibers with aging, as well as to establish mutual interactions and correlations with age. Material was samples of hypophysis and psoas major muscle of 27 cadavers of both genders, aged from 30 to 90 years. Adenohypophyseal and psoas major tissue sections were immunohistochemically processed and stained by anti-human growth hormone and anti-fast myosin antibodies, respectively. Morphometric analysis was performed by ImageJ. Results of morphometric analysis showed a significant increase in the somatotrope area, and significant decrease in somatotrope volume density and nucleocytoplasmic ratio with age. Cross-sectional areas of types 1 and 2, and volume density of type 2 muscle fibers decreased significantly with age. One Way ANOVA showed that the latter cited changes in the somatotropes and types 1 and 2 muscle fibers mostly become significant after the age of 70. Significant positive correlation was observed between the area of the somatotropes and volume density of type 2 muscle fibers. A significant negative correlation was detected between the nucleocytoplasmic ratio of the somatotropes and cross-sectional areas of types 1 and 2 muscle fibers. So, it can be concluded that after the age of 70, there is significant loss of the anterior pituitary's somatotropes associated with hypertrophy and possible functional decline of the remained cells. Age-related changes in the somatotropes are correlated with the simultaneous atrophy of type 1, as well as with the atrophy and loss of type 2 muscle fibers.

1. Introduction

The involuntary loss of muscle mass, strength and function that occurs with age is termed sarcopenia. Muscle mass starts to decrease after the age of 30 and this decline becomes even greater after the age of 60 causing and contributing to functional dependence and disability in older people. This has major healthcare as well as socioeconomic implications, such as development of frailty, associated morbidity, increased hospitalisation and mortality (Goldspink, 2004; Mühlberg and Sieber, 2004; Volpi et al., 2004). The etiology of sarcopenia is not clearly understood, but most likely it represents a multifactorial problem. Physical inactivity, smoking and poor diet, age-related changes in cytokine levels, increased oxidative stress, loss of alpha-motor neurons, muscle cell apoptosis and genetic susceptibility are important risk factors. A variety of hormonal changes that can be seen during the aging process may contribute to muscle loss, too. There is evidence linking insulin, estrogens, androgens, prolactin, thyroid hormones, catecholamines and corticosteroids to the etiology and pathogenesis of sarcopenia (Kamel et al., 2002; Solomon and Bouloux, 2006; Giovannini et al., 2008; Rolland et al., 2008). Insulin, growth hormone (GH), insulin-like growth factor-1 (IGF-1) and testosterone are major anabolic hormones which increase muscle mass. Circulating levels of the above cited hormones vary during the aging process potentially contributing to the development of sarcopenia (Marcell et al., 2001). Decreased levels of testosterone in elderly

* Corresponding author. Tel.: +381 18 510 900; fax: +381 18 242 482.
E-mail address: vlada.antic@hotmail.com (V.M. Antić).

http://dx.doi.org/10.1016/j.aanat.2015.01.006
0940-9602/

(andropause), gradual decrease in dehydroepiandrosterone with age (adrenopause) and, impaired ability of the muscle cells to properly respond to circulating insulin may cause a decline in muscle protein synthesis and consequent loss of muscle mass and strength (Mühlberg and Sieber, 2004; Volpi et al., 2004; Giovannini et al., 2008; Sattler, 2013). Growth hormone elicits anabolic effects in the skeletal muscles through the hepatic production and release of IGF-1 (circulating or endocrine IGF-1) (Le Roith et al., 2001; Melmed et al., 2011), local production of mechanogrowth factor (MGF, IGF-1Ec) and IGF-1Ea (Giovannini et al., 2008; Sattler, 2013) and, reduced expression of myostatin, an inhibitor of muscle growth and promoter of adipogenesis (Marcell et al., 2001; Goldspink, 2004; Solomon and Bouloux, 2006; Perrini et al., 2010; Melmed et al., 2011; Puche and Castilla-Cortázar, 2012). Circulating and locally produced IGF-1 mediate muscle growth through the enhanced delivery of amino acids, suppression of proteolysis and upregulation of protein synthesis, attenuation of inflammation and fibrosis after vigorous muscle contraction and activation of proliferation and differentiation of satellite cells (Liu et al., 2006; Sattler, 2013). The GH/IGF-1 axis described above also exhibits a gradual decline during normal aging. This process is known as somatopause and is associated with the detrimental changes in body composition; i.e. reduction in lean body mass, increased adiposity and decline of the muscle mass and strength (Mühlberg and Sieber, 2004; Volpi et al., 2004; Giovannini et al., 2008; Perrini et al., 2010; Sattler, 2013).

Despite the fact that no other treatment of sarcopenia has proven to be as efficacious as resistance training, resistance training sessions and programs might be very challenging in frail elderly subjects. Also, there are conflicting and inconclusive results about the effectiveness of protein, vitamin D and creatine supplementation, then the application of testosterone, estrogens, tibolone, ACE inhibitors and cytokine inhibitors, while administration of caspase inhibitors and antagonists of myostatin represent a possible future therapy for the improvement of the muscle mass and muscle strength in elderly. Beyond the above cited potential modalities of treatment for sarcopenia, age-related changes in endocrine function are potentially treatable with pharmacological agents. Several studies have demonstrated that testosterone replacement therapy could be a useful intervention in hypogonadal older men for increasing muscle mass and strength. Hormone replacement therapy for adrenopause appears to have marginal or no positive effect on muscle mass and strength (Volpi et al., 2004; Rolland et al., 2008). Opinions about the potential use of GH, GH secretagogues and IGF-1 in treatment of sarcopenia are highly controversial based on to data from clinical studies indicating an unfavorable risk-benefit ratio in endocrinologically normal elderly subjects (Bartke, 2009). Administration of GH alone or in combination with IGF-1 in the elderly appears to be beneficial to increasing muscle mass, lowering fat mass, improving blood lipid profiles and increasing lean body mass. Nevertheless, these changes may not lead to an increase in muscle strength and function. Muscle strength increased in cases in which GH was given to elderly men in combination with a weight-training program, or with sex hormone replacement therapy (Volpi et al., 2004). Muscles as a dynamic metabolic store of the human body are important in maintaining boney tissue, body temperature and production of proteins in preventing tissue cachexia and providing metabolites required for acid-base balance in traumatic situations in elderly (Goldspink, 2004; Rolland et al., 2008). So, despite the absence of increase in muscle strength, an increase in muscle mass is one more important aspect of the application of GH in sarcopenic elderly individuals for the above cited reasons and deserves further study.

The majority of the studies consider that age-dependent decline in GH secretion is the consequence of the changes that occur at the hypothalamic level. So, they assume that unknown factors cause a decrease in growth hormone–releasing hormone (GHRH), and an increase in somatostatin secretion, which secondarily causes decreased secretion of GH from the anterior pituitary (Perrini et al., 2010). However, according to the Shimokawa et al. (1996) and Kamel et al. (2002) significant changes are also found in the responsiveness of the pituitary gland to GHRH with decreased sensitivity of the somatotropes to GHRH in aged organisms. Generally, there is a lack of morphometric data on the age-related alterations in the anterior pituitary's somatotropes. Also, the data which could illustrate the possibility of whether these changes with age are accompanied by alterations in the number and the size of different types of muscle fibers are even more limited.

So, the aim of our research was to immunohistochemically and morphometrically analyze changes of the anterior pituitary somatotropes with aging, as well as to establish to what extent these changes are associated with corresponding changes of types 1 and 2 muscle fibers of the psoas major muscle. In this manner, the results of our study may contribute to further clarification of the endocrine underpinnings of the loss, changes in grouping, atrophy of different types of the muscle fibers, and subsequent development of sarcopenia in the elderly. Also, this might be important for the eventual further possible application of human GH in the treatment of such conditions in the future.

2. Materials and methods

2.1. Material

The pituitary gland and tissue samples of the right psoas major muscle of 27 adult cadavers (13 males and 14 females) were obtained post mortem at autopsies performed at the Institute of Forensic Medicine, Faculty of Medicine in Niš during the period of January–April 2013. The age of the cadavers ranged from 30 to 90 years. Based on the available medical documentation, the cadavers included in the study had not suffered from any neurological or neuromuscular disorders during the lifetime. Results of the autopsies confirmed the absence of pathological changes of the brain and psoas major muscle in analyzed cases. The period from time of death to sampling was no longer than 24 h. The tissue samples used in this study were obtained according to the rules of the faculties Internal Ethical Committee. Cadavers were classified into three age groups: the first group (I) ranged from 30 to 49 years of age, the second group (II) included the cases from 50 to 69 years of age and the third group (III) included cases 70 years and older.

2.2. Dissection procedures

First, the gland was separated from the hypothalamus by cutting the pituitary stalk. Afterwards, the diaphragma sellae was removed, the gland separated from the osseous structures of the sella turcica and taken en bloc. Samples of the psoas major muscle were obtained from the middle region of the muscle using an incision made perpendicular to the longitudinal axis of the muscle. Pituitary gland and psoas major muscle tissue samples were then fixed in 10% buffered formalin for 24 h. After that, the pituitary gland was horizontally cut at the middle of the distance between its dorsal and ventral surfaces, perpendicular to its dorsoventral axis. The ventral half of the gland was used for further histological processing. Psoas major muscle samples were additionally dissected into $5 \times 5 \times 5$ mm large samples which were subsequently histologically processed.

2.3. Immunohistochemistry

Tissue samples of the hypophysis and psoas major muscle were embedded in paraffin and cut into 5 μm thick sections using a