Experimental Gerontology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

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

Study of insulin vascular sensitivity in aortic rings and endothelial cells from aged rats subjected to caloric restriction: Role of perivascular adipose tissue

S. Amor[a], B. Martín-Carro[a], C. Rubio[b], J.M. Carrascosa[b], W. Hu[c], Y. Huang[c], A.L. García-Villalón[a], M. Granado[a,d,*]

[a] Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, Spain
[b] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Madrid, Spain
[c] School of Biomedical Sciences, Institute of Vascular Medicine, Faculty of Medicine, Chinese University of Hong Kong, China
[d] CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain

ARTICLE INFO

Keywords:
Aging
Insulin
Akt
Perivascular adipose tissue
Aorta
eNOS

ABSTRACT

The prevalence of metabolic syndrome is dramatically increasing among elderly population. Metabolic syndrome in aged individuals is associated with hyperinsulinemia and insulin resistance both in metabolic tissues and in the cardiovascular system, with this fact being associated with the cardiometabolic alterations associated to this condition. Caloric restriction (CR) improves insulin sensitivity and is one of the dietetic strategies most commonly used to enlarge life and to prevent aging induced cardiovascular alterations. The aim of this study was to analyze the possible beneficial effects of CR in aging-induced vascular insulin resistance both in aortic rings and in primary culture of endothelial cells. In addition, the inflammatory profile of perivascular adipose tissue (PVAT) and its possible role in the impairment of vascular insulin sensitivity associated with aging was also assessed. Three experimental groups of male Wistar rats were used: 3 (3 m), 24 (24 m) fed ad libitum and 24 months old rats subjected to 20% CR during their three last months of life (24 m–CR). Aorta rings surrounded or not by PVAT were mounted in an organ bath and precontracted with phenylephrine ($10^{-7.5}$ M). Changes in isometric tension were recorded in response to cumulative insulin concentrations (10^{-8}–$10^{-5.5}$ M) in the presence or absence of L-NAME (10^{-4} M). Aortic rings and primary aortic endothelial cells were incubated in presence/absence of insulin (10^{-7} M) and the activation of the PI3K/Akt and MAPK pathways as well as nitrite and nitrates concentrations and the mRNA levels of eNOS, insulin receptor, and GLUT-4 were assessed. CR prevented the aging-induced decrease in the vasodilator response to insulin and the aging-induced increase in the vasoconstrictor response to high insulin concentrations. Changes between 24 m and 24 m–CR aorta rings were abolished in the presence of L-NAME. CR induced-improvement in insulin vascular sensitivity was related with activation of the PI3K/Akt both in aortic rings and in aortic endothelial cells in response to insulin. CR attenuated the overexpression of iNOS, TNF-α and IL-1β in the PVAT of aged rats although aortic rings surrounded by PVAT from 24 m rats showed and increased vasorelaxation in response to insulin compared to aortic rings from 3 m and 24 m–CR rats. In conclusion, a moderate protocol of CR improves insulin vascular sensitivity and prevents the aging induced overexpression of pro-inflammatory cytokines in PVAT.

1. Introduction

Aging is the major risk factor for cardiovascular diseases, as nearly 90% of incident CV events occur in adults over 55 years of age (Gu and Xu, 2013). Aging is associated with several cardiovascular alterations such as endothelial dysfunction and arterial stiffness that lead to an impairment on cardiovascular function (Tuomilehto, 2004). Some of these alterations are linked to metabolic syndrome whose prevalence is increased among elderly men and women. Indeed, the ratio for metabolic syndrome among men and women of 65 years old of age and older is approximately fivefold higher than among those aged 20–34 years old (Park et al., 2003; Schulman et al., 2007).

A large body of evidence demonstrates that aging and metabolic syndrome share several metabolic alterations that include an altered distribution, expansion, and endocrine function of adipose tissue (Bonomini et al., 2015; Haffner, 2000), as well as hyperinsulinaemia and insulin resistance (Armani et al., 2017). Indeed, the onset of insulin resistance and type 2 diabetes is a hallmark of aging (Lopez-Lluch and

* Corresponding author at: Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, C/Arzobispo Morcillo no. 2, 28029 Madrid, Spain.
E-mail address: miriam.granado@uam.es (M. Granado).

http://dx.doi.org/10.1016/j.exger.2017.10.017
Received 4 August 2017; Received in revised form 15 October 2017; Accepted 16 October 2017

Navas, 2016). Aging-induced alterations in cardiovascular function are related to impairment in insulin sensitivity both in metabolic tissues (Escriva et al., 2007) and in the cardiovascular system (Boudina, 2013), where insulin exerts direct effects in physiological conditions, both in the heart and in the vasculature (Muniyappa et al., 2007). In the myocardium insulin increases heart contractility (positive inotropic effect) through activation of Ca channels both in vivo (von Lewinski et al., 2005) and in vitro (Maier et al., 1999). In the vasculature insulin exerts vasodilatory actions through the activation of the insulin receptor (IR) tyrosine kinase, leading to tyrosine phosphorylation of IR substrate (IRS)-1, which binds and activates phosphatidylinositol 3-kinase (PI3K). PI3K phosphorylates Akt at serine 473, which directly activates endothelial nitric oxide (NO) synthase (eNOS) via phosphorylation at serine 1177. In blood vessels, the activation of the PI3K/Akt pathway in response to insulin leads, in the first place, to an increase in blood flow via capillary recruitment and, in the second place, to the vasodilation of larger blood vessels (Dimmeler et al., 1999; Steinberg et al., 1994). The impairment of insulin signaling in the vasculature is related to a reduced activation of the PI3K/Akt pathway which induces a decrease in NO bioavailability and an increase in the adhesion of mononuclear cells to the endothelium which, in the end, accelerate the atherosclerotic process and promotes a deleterious effect in cardiovascular function (Rask-Madsen et al., 2010). Interestingly, it is reported that in situations of hyperinsulinaemia and vascular insulin resistance there is a selective inhibition of the PI3K/Akt pathway whereas the activation of the mitogen-activated protein kinases (MAPKs) pathway, which mediates the proliferative and vasoconstrictor effects of insulin through the production of endothelin-1 (ET-1), remains unaffected (Cusi et al., 2000; Jiang et al., 1999; Muniyappa et al., 2007). Thus, the antihypertensive effects of insulin mediated by NO production are reduced under conditions of insulin resistance.

Vascular insulin resistance in a context of metabolic syndrome may be linked, at least in part, to the secretion of proinflammatoy mediators by perivascular adipose tissue, since it is reported that in the metabolic syndrome this tissue becomes highly inflamed and induces vascular dysfunction through an augmented secretion of pro-inflammatory adipokines and vasoconstrictive factors such as the components of renin-angiotensinogen-aldosterone system and reactive oxygen species (Szasz et al., 2013; Szasz and Webb, 2012).

Caloric restriction (CR) is a dietary intervention that delays aging and extends lifespan in diverse species (Anderson and Weindruch, 2010) due, at least in part, to a promotion in cardiovascular health (Lopez-Lluch and Navas, 2016). Moreover, CR with adequate nutrition improves several deleterious conditions present in elder individuals such as insulin resistance, increased fasting glucose and insulin concentrations and prevents obesity, type 2 diabetes, hypertension and chronic inflammation (Soare et al., 2014). Thus, CR is considered a promising strategy to treat/prevent the metabolic and cardiovascular alterations associated with both aging and metabolic diseases. However the severity of CR plays a major in role in its beneficial effects (Vitousek et al., 2004), being reported in rodents that a protocol of 20% reduction in food intake for 3 months is effective decreasing adiposity, improving insulin sensitivity, both centrally and peripherally (Escriva et al., 2007; García-San Frutos et al., 2007), and preventing some of the aging induced alterations in cardiovascular function (Amor et al., 2017; Granado et al., 2014). However it is unknown weather this specific protocol of moderate CR is able to modify the inflammatory profile of perivascular adipose tissue and to prevent the aging-induced alterations in vascular insulin sensitivity in rats. Therefore the aim of this work was to analyze insulin vascular sensitivity in aortic rings and vascular endothelial cells from aged rats subjected to caloric restriction, as well as the possible role of perivascular adipose tissue in this response.

2. Material and methods

2.1. Animals

Three (n = 12) and 24-months-old (n = 24) male Wistar rats from our in-house colony (Centre of Molecular Biology, Madrid, Spain) were used throughout this study. Rats were housed in climate-controlled quarters with a 12 h light cycle and fed ad libitum a standard laboratory chow A04–10 Rodent Maintenance Diet (SAFE, Spain) and water. Handling of animals was performed according to European Union laws and the guidelines of the National Institutes of Health). Experimental procedures were approved by the Institutional Committee of Research Ethics.

2.2. Caloric restriction

Half of the 21-months-old rats (n = 12) were assigned to undergo a caloric restriction protocol as previously described (Perez et al., 2004). Animals were placed in individual cages and fed daily an amount of chow equivalent to 80% of normal food intake without supplementation with additional minerals or vitamins. Caloric restriction was prolonged during three months.

2.3. Experiments of vascular reactivity

Restricted (CR) and ad libitum fed rats were decapitated at the ages of 3 (3 m) and 24 (24 m) months old previously anesthetized with sodium pentobarbital (100 mg/kg i.p.). Immediately after death, aorta was carefully dissected, cut in 2 mm segments and kept in cold isotonic saline solution. Each segment was mounted in a 4 ml organ bath containing modified Krebs–Henseleit solution at 37 °C (mM): NaCl, 115; KCl, 4.6; KH2PO4, 1.2; MgSO4, 1.2; CaCl2, 2.5; NaHCO3, 25; glucose, 11. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to a pH of 7.3–7.4. Briefly, two fine steel wires (100 μm diameter) were passed through the lumen of the vascular segment. One wire was fixed to the organ bath wall and the other wire was connected to a strain gauge for isometric tension recording (Universal Transducing Cell UC3 and Statham Microscale Accessory UL5, Statham Instruments, Inc.). This arrangement permits passive tension to be applied in a plane perpendicular to the long axis of the vascular cylinder. The changes in isometric force were recorded using a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO, USA). After applying an optimal passive tension of 1 g, vascular segments were allowed to equilibrate for 60–90 min. Afterwards, segments were stimulated with potassium chloride (KCl 100 mM) to determine the contractility of smooth muscle. Segments which failed to contract at least 0.5 g to KCl were discarded. After equilibration, the segments were precontracted with $10^{-7,5}$M phenylephrine (Sigma-Aldrich, St. Louis, MO, USA) to subsequently perform a cumulative dose-response curve in response to insulin (10^{-8}–$10^{-5.5}$ M) (Sigma-Aldrich, St. Louis, MO, USA). The relaxation in response to insulin was determined based on the percentage of the active tone achieved by the NO donor sodium nitroprusside (10^{-5} M) (Sigma-Aldrich, St. Louis, MO, USA). The highest concentrations of insulin in the dose-response curve induced contraction instead of vasodilation in some aorta segments. This contraction was measured as the increase in tension over the level reached at the end of the vasodilation, and expressed as percentage of the contraction to potassium chloride (100 mM).

To study the mechanism of the vasodilation in response to insulin some segments were preincubated for 30 min with the inhibitor of the nitric oxide synthase L-NAME (10^{-4} M) (Nω-Nitro-L-arginine methyl ester hydrochloride) (Sigma-Aldrich, St. Louis, MO, USA). Moreover, to study the mechanism of vasoconstriction in response to high doses of insulin other segments were preincubated for 30 min with the antagonist of the angiotensin II receptor type 1 Losartan (10^{-5} M) (2-Butyl-4-chloro-1-[2′-(1H–tetrazol-5-yl)(1,1′-biphenyl)-4-yl]methyl 1H-