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Evidence for association of mitochondrial metabolism alteration with lipid accumulation in aging rats

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ABSTRACT

Adipogenesis and lipid accumulation during aging have a great impact on the aging process and the pathogenesis of chronic, age-related diseases. However, little is known about the age-related molecular changes in lipid accumulation and the mechanisms underlying them. Here, using 5-month- and 25-month-old rats (*young* and *old*, respectively), we found that epididymal fat is the only tissue to accumulate during aging. By testing tissues rich with mitochondria in old and young animals, we found that the old animals had elevated levels of triglycerides in their muscle, heart and liver tissues but not in their kidneys, while, the mRNA level of fatty acid synthase remained unchanged among the four tissues. Regarding lipid degradation, we determined that the activities of mitochondrial ETC. complexes changed in aged rats (muscle: decreased complex I and V activities; heart: decreased complex I activity; liver: increased complex I and III activities; kidney: decreased complex I and increased complex II activities), while changes in mitochondrial content were not observed in the muscle, heart nor in the liver tissue except increased complex IV and V subunits in aged kidneys. Furthermore, decreased mitochondrial fusion marker Mfn2 and decreased PGC-1α level were observed in the aged muscle, heart and liver but remained unchanged in the kidneys. Down-regulation of Mfn2 with siRNA in 293T cells induced significant mitochondrial dysfunction including decreased oxygen consumption, decreased ATP production, and increased ROS production, followed by increased triglyceride content suggesting a contributing role of decreased mitochondrial fusion to lipid deposit. Meanwhile, judging from autophagy marker p62/SQSTM1 and LC3-II, autophagy was suppressed in the aged muscle, heart and liver but remained unchanged in the kidneys. Taken together, these data suggest that reduction in PGC-1α expression and disruption of mitochondrial dynamics and autophagy might contribute to lipid accumulation during aging.

1. Introduction

Fat tissue is involved with nutrient storage, endocrine function and immunity and undergoes renewal throughout the lifespan of an adult. As the largest organ in humans, fat tissue is thought to play a role in longevity, the progression of age-related disease, inflammation, and metabolic dysfunction (Tchkonia et al., 2010). Total adiposity is usually found to increase during an adult's lifespan. For example, compared with young adult Caucasian men, older men were found to have increased adiposity according to measurements of body mass index or total body fat mass (Couillard et al., 2000). In middle-aged women, adipose tissue accumulation is increased and contributes to the deterioration of cardiovascular disease risk profiles (Pascot et al., 1999). Generally, adipose tissue is located beneath the skin and around vital organs. During aging, there is an age-related decline in subcutaneous adipose depot size but very late decline in visceral depot size. The age-related loss of subcutaneous fat is accompanied by increased accumulation of fat in the bone marrow, muscle, liver, and other ectopic sites (Cartwright et al., 2007; Kuk et al., 2009). These changes in fat deposition are associated with health risks; it has been shown that excessive fat deposits in the liver increase the incidence of diabetes and cardiovascular disease in middle-aged, non-diabetic subjects (Gastaldelli et al., 2009). The realization that lipid accumulation contributes to the development of health risk factors indicates the importance of lipid

Abbreviations: SREBP1, Sterol regulatory element-binding protein-1; FAS, Fatty acid synthase; CPT1A, Carnitine palmitoyltransferase IA; CPT1B, Carnitine palmitoyltransferase IB; TG, Triglyceride.

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metabolism throughout human life. By targeting lipid metabolism with interventions like exercise (DiPietro, 2010; Rosa et al., 2005) or caloric restriction (Hansen, 2001), age-associated metabolic status and physical function can be improved.

Although studies of lipid metabolism have been carried out for decades and several processes have been reported to be involved in its regulation, the mechanisms underlying lipid accumulation during aging are still poorly understood. Lipid homeostasis is normally controlled by sterol regulatory element-binding proteins (SREBPs). The SREBPs transcriptionally activate an enzyme cascade required for the synthesis of endogenous cholesterol, fatty acids, triglycerides (TGs) and phospholipids (Eberle et al., 2004). It has been reported that the SREBP-1-mediated regulation of lipogenesis is highly involved in the development of fatty livers (Yahagi et al., 2002) and may also be responsible for lipid accumulation in the muscle (Ikeda et al., 2002; Nadeau et al., 2006). Regarding lipid degradation, nutrient depletion leads to the mobilization of cellular lipid stores to supply free fatty acids for energy, suggesting that there are regulatory and functional similarities between autophagy and lipid metabolism (Singh, 2010; Singh et al., 2009). Inhibition of autophagy decreases triglyceride breakdown, leading to increases in triglyceride levels and lipid droplets *in vitro* and *in vivo* (Singh et al., 2009). Genetic inhibition of autophagy in mammalian tissues may induce degenerative changes resembling those associated with aging, and normal and pathological aging is often associated with a reduction in autophagic potential (Rubinsztein et al., 2011). However, aging is a complex process, and the relationship between autophagy and lipid metabolism during aging requires further study.

The mitochondria are the organelles that provide the cell with energy and are involved in several diseases and the aging process. The dynamic character of these organelles involves mitochondrial biogenesis and frequent fusion and fission events (Kowald and Kirkwood, 2011). It has been widely accepted that PGC-1α stimulates the efficient induction of NRF-1 and NRF-2 gene expression and binds the promoter of mitochondrial transcription factor A (mtTFA) to regulate mitochondrial biogenesis and fatty acid oxidation (Vega et al., 2000; Wu et al., 1999). While the study of mitochondrial dynamics has received attention in recent years, the mechanisms underlying mitochondrial fusion and fission are still poorly understood. Thus far, studies have suggested that Fis1 and Drp1 are involved in the mitochondrial fission machinery, and OPA, Mfn1, and Mfn2 contribute to the regulation of mitochondrial fusion (Chen and Chan, 2005; Song et al., 2009). The disruption of mitochondrial fusion by the knockdown of mitofusins (Mfns) or OPA1 leads to mitochondrial fragmentation and the accumulation of TGs in adipocytes, suggesting a close connection between the regulation of mitochondrial dynamics and lipid metabolism (Kita et al., 2009). The effect that the remodeling of mitochondrial dynamics may have on lipid metabolism during aging is poorly understood. Therefore, in our present study, we focused on tissues rich in mitochondria—including muscle, heart, liver and kidney tissues—and determined that TGs accumulate in aged muscle, heart and liver tissues but not in the kidneys; this accumulation was accompanied by autophagy inactivation, mitochondrial dynamic alteration and a decrease in PGC-1α expression. Therefore, we propose that mitochondrial metabolism and autophagy might all be involved in lipid accumulation during aging.

2. Materials and methods

2.1. Reagents

Cytochrome c, coenzyme Q_1, $NADP^+$, antimycin A and dithiothreitol were purchased from Sigma Chemical Co. (St. Louis, MO); Tris base and NADH from Amersco, Inc. (Palm Harbor, FL); 2,6-dichlorophenol indophenol (DCPIP) from Merck & Co., Inc.; rotenone from Riedel De Haen Seelze (Hannover, Germany); antibodies to CPT1A, CPT1B, LC3, Mfn-1, Mfn-2, Drp-1, OPA-1 and PGC-1α from Santa Cruz Biotechnology (Santa Cruz, CA); anti-GAPDH from Cell Signaling Technology (Danvers, MA); and antibodies to complexes I (NDUFS3), II (subunit 30 kDa), III (subunit core 2), IV (subunit I), and V (subunit alpha) from Invitrogen (Carlsbad, CA). Other chemicals and reagents were purchased from Sigma if not otherwise indicated.

2.2. Animals

Sprague–Dawley (SD) male rats were purchased from a commercial breeder (SLAC, Shanghai). The rats were housed in a temperature- (22–28 °C) and humidity- (60%) controlled animal room and maintained on a 12-h light/12-h dark cycle (light on from 08:00 a.m. to 08:00 p.m.) with free access to food and water throughout the experiments. Four-week-old male rats weighing 180–200 g were used to start the experiments. After reaching 25 months and 5 months of age (old and young groups, respectively), the animals were sacrificed and various tissue samples were collected and weighed.

2.3. Isolation of muscle, heart and kidney mitochondria

At the time of sacrifice and after the tissue weight measurements were taken, the bulk of the skeletal muscle, heart and kidney tissues was collected and frozen in liquid N_2. A small portion of fresh tissue from each organ was used to isolate mitochondria as previously described (Shen et al., 2008). Briefly, tissues were trimmed of fat and connective tissue, chopped finely with a pair of scissors, and rinsed in ice-cold medium A (120 mM NaCl, 20 mM HEPES, 2 mM $MgCl_2$, 1 mM EGTA, and 5 g/l bovine serum albumin; pH 7.4) to remove any residual blood. The chopped tissues were resuspended in medium A and homogenized with a hand-held borosilicate glass homogenizer. The homogenate was centrifuged at 600 *g* for 10 min at 4 °C. The supernatant fluid was subsequently recentrifuged at 17,000 *g* for 10 min at 4 °C. The pellet containing the mitochondria was resuspended in medium A and then centrifuged at 7000 *g* for 10 min at 4 °C. The pellet obtained after the last centrifugation was resuspended in medium B (300 mM sucrose, 2 mM HEPES, 0.1 mM EGTA; pH 7.4) and recentrifuged (3500 *g*, 10 min, 4 °C). The resulting pellet, which contained skeletal muscle, heart or kidney mitochondria, was suspended in a small volume of medium B and stored at −70 °C.

2.4. Isolation of liver mitochondria

At the time of sacrifice and after the liver weight measurements were taken, the bulk of the liver was collected and frozen in liquid N_2. A small fresh portion was used to isolate mitochondria as previously described (Sun et al., 2010). Briefly, the tissues were rinsed with saline, weighed, and put into an ice-cold isolation buffer containing 0.25 M sucrose, 10 mM Tris, and 0.5 mM EDTA at pH 7.4. The tissues were minced by careful shearing, rinsed to remove residual blood, and then homogenized in the isolation buffer. The homogenate was centrifuged at 1000 *g* for 10 min; the supernatant was then centrifuged at 10,000 *g* for 10 min. The mitochondrial pellet was collected and washed twice and resuspended in the isolation buffer. The mitochondrial protein concentrations were determined using a BCA Protein Assay kit (Pierce, IL). The pellets were stored at −70 °C. All of the operations were carried out at 4 °C.

2.5. Assays for mitochondrial complex activities

NADH–ubiquinone reductase (complex I), succinate–CoQ oxidoreductase (complex II), ubiquinol cytochrome c reductase (complex III), cytochrome c oxidase (complex IV) and Mg^{2+}–ATPase (complex V) were measured spectrometrically using conventional assays as described (Long et al., 2006; Sun et al., 2006).

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