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Dual regulation of adipose triglyceride lipase by pigment epithelium-derived factor: A novel mechanistic insight into progressive obesity

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ABSTRACT

Both elevated plasma free fatty acids (FFA) and accumulating triglyceride in adipose tissue are observed in the process of obesity and insulin resistance. This contradictory phenomenon and its underlying mechanisms have not been thoroughly elucidated. Recent studies have demonstrated that pigment epithelium-derived factor (PEDF) contributes to elevated plasma FFA and insulin resistance in obese mice via the activation of adipose triglyceride lipase (ATGL). However, we found that PEDF downregulated adipose ATGL protein expression despite of enhancing lipolysis. Plasma PEDF and FFA were increased in associated with a progressive high-fat-diet, and those outcomes were also accompanied by fat accumulation and a reduction in adipose ATGL. Exogenous PEDF injection downregulated adipose ATGL protein expression and elevated plasma FFA, while endogenous PEDF neutralization significantly rescued the adipose ATGL reduction and also reduced plasma FFA in obese mice. PEDF reduced ATGL protein expression in a time- and dose-dependent manner in differentiated 3T3-L1 cells. Small interfering RNA-mediated PEDF knockdown and antibody-mediated PEDF blockage increased endogenous ATGL expression, and PEDF overexpression downregulated ATGL. PEDF resulted in a decreased half-life of ATGL and regulated ATGL degradation via ubiquitin-dependent proteasomal degradation pathway. PEDF stimulated lipolysis via ATGL using ATGL inhibitor bromoenol lactone, and PEDF also downregulated G0/G1 switch gene 2 (G0S2) expression, which is an endogenous inhibitor of ATGL activation. Overall, PEDF attenuated ATGL protein accumulation via proteasome-mediated degradation in adipocytes, and PEDF also promoted lipolysis by activating ATGL. Elevated PEDF may contribute to progressive obesity and insulin resistance via its dual regulation of ATGL.

1. Introduction

Adipose tissue represents an individual's pool of excess energy that is stored in the form of triglycerides deposited in lipid droplets. During starvation and exercise, adipose tissue mobilizes to meet the energy demands of the body by hydrolyzing triglycerides into free fatty acids (FFA) and glycerol (Rosen and Spiegelman, 2006). Over the past several years, adipose tissue has been emphasized as the master regulatory organ that controls the whole-body lipid flux. Deregulation of adipose tissue function may lead to excessive circulating FFA and ectopic fat accumulation in non-adipose tissues, such as the liver and skeletal muscle, which results in obesity and type 2 diabetes (Guilherme et al., 2008; Rosen and Spiegelman, 2006; Savage et al., 2007). Hence, studies of adipose lipid regulation may yield treatments for obesity and type 2 diabetes.

Lipolysis is a catabolic process that breaks down triglycerides stored in adipose tissue and releases non-esterified fatty acid and glycerol, and the process is precisely controlled by a number of enzymes and factors. For several decades, hormone-sensitive lipase (HSL) was believed to catabolize the initiate cleavage of triglycerides, until adipose triglyceride lipase (ATGL) was identified as a lipase that hydrolyzes triglycerides by three different groups independently (Jenkins et al., 2004; Villena et al., 2004; Zimmermann et al., 2004). It became clear that ATGL firstly hydrolyzes triglycerides, thereby initiating the degradation of stored lipids. Then, diglycerides are cleaved by HSL, and monoglyceride lipase contributes to the final step of lipolysis (Duncan et al., 2007). Moreover, *in vivo* studies have demonstrated that ATGL-deficiency mice exhibit adiposity and impaired lipolysis both in basal and isoproterenol

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(ISO)-stimulated states, as well as reduced plasma FFA levels (Zimmermann et al., 2004). Human subjects with mutated ATGL developed a neutral lipid storage disease (NLSD) (Fischer et al., 2006), which indicated that ATGL is the rate-limiting enzyme that controls the release of cellular FFA. ATGL may be a drug target based on the evidence that inhibition of ATGL reduces the concentration of plasma FFA, which increases insulin sensitivity (Zimmermann et al., 2009).

Both plasma FFA and cellular FFA levels are positively correlated with increased insulin resistance and obesity (Boden and Shulman, 2002; Gordon, 1960; Nielsen et al., 2004). Elevated plasma FFA comes from enhancing basal lipolysis in obesity. However, downregulation of adipose ATGL, the key player in lipolysis, is often observed in obese and insulin resistance humans (Jocken et al., 2007; Steinberg et al., 2007) and rodents (Kim et al., 2006). The reasons for these paradoxical phenomena remain unknown, and it is unclear why adipose ATGL levels are not always positively associated with plasma FFA in obesity. Investigation of the mechanism of decreased adipose ATGL and elevated plasma FFA will help us to understand the discrepancy.

Despite sequestering lipids, adipose tissue also regulates body energy homeostasis through the secretion of cytokines or adipokines, such as adiponectin and leptin (Guilherme et al., 2008; Halaas et al., 1995; Maeda et al., 2002), which improve insulin sensitivity. However, in obesity, adipocytes also secrete adipokines that contribute to the development of insulin resistance and obesity, including tumor necrosis factor-α (TNF-α) (Hotamisligil, 1999; Miyazaki et al., 2003), monocyte chemoattractant protein-1 (Sartipy and Loskutoff, 2003), resistin (Steppan and Lazar, 2004), and retinol-binding protein (Yang et al., 2005). PEDF is a well-known inhibitor of angiogenesis (Dawson et al., 1999; Gao et al., 2001), as well as a neuroprotective factor (Tombran-Tink and Barnstable, 2003) and an anti-tumor factor (Fernandez-Garcia et al., 2007; Xu et al., 2011; Yang et al., 2009; Zhang et al., 2011). Clinical studies have elucidated that plasma PEDF is elevated in patients who are obese (Wang et al., 2008), and who have metabolic syndrome (Yamagishi et al., 2006) and type 2 diabetes (Akın et al., 2012). Further studies showed that PEDF inhibited adipogenesis via inhibition of the MAPK/ERK pathway and mitotic clonal expansion (Wang et al., 2009). Recent work reported that PEDF released from adipose tissue contributed to the pathogenesis of insulin resistance in obesity and also enhanced adipocyte lipolysis (Crowe et al., 2009). In addition to governing lipolysis in adipocytes, ATGL has been shown to bind with PEDF in the plasma membrane of the retina as a receptor (Notari et al., 2006) and to interact with PEDF in hepatocytes (Chung et al., 2008). Another study also showed that PEDF induces lipolysis in adipocytes and reduces fatty acid oxidation in skeletal muscle in an ATGL-dependent manner (Borg et al., 2011).

Our study elucidated that elevated adipose PEDF decreases ATGL protein expression in adipose tissue in obesity, as well as induces basal lipolysis, which explains the concurrent phenomena of progressive fat accumulation and enhanced lipolysis in obesity.

2. Materials and methods

2.1. Ethics statement

Care, use and treatment of all animals in the present study were in strict agreement with the institutionally approved protocol that followed the guidelines set forth in the Care and Use of Laboratory Animals by the Sun Yat-sen University. The experiment procedures were reviewed and approved by the institutional animal care and use committee of Sun Yat-sen University (IACUC SYSU, NO. 20061211005).

2.2. Experimental animals and protocols

Male C57BL/6J mice (eight-weeks-old) were obtained from the Center of Experimental Animals, Sun Yat-sen University (Guangzhou, China). For the HFD-induced obese mouse model, mice ($n = 6$) were allowed to acclimate to local conditions for 1 week and then were fed a normal chow diet or a HFD (60% calories from fat; animal center of Guangdong Province, Guangzhou, China) for 4 weeks, 8 weeks, 12 weeks or 16 weeks (Crowe et al., 2009). Mice were anesthetized by intraperitoneal injection of urethane after 8 h of fasting, and they were subsequently sacrificed for tissues and plasma collection. For the *in vivo* experiment of prolonged PEDF administration, PEDF protein (primary concentration: 7.5 mg/ml) was diluted with PBS to 1 mg/ml prior to injection. Mice ($n = 8$) were allowed ad libitum access to food and were injected intraperitoneally with 1 × PBS or PEDF daily (50 μg; totally 50 μl) for 7 days, and they were sacrificed for tissue and plasma collection after 8 h of fasting (Borg et al., 2011; Crowe et al., 2009). For the *in vivo* experiment of acute PEDF administration, mice ($n = 5$) were injected with 1 × PBS or PEDF (50 μg) prior to 8 h of fasting, and they were then maintained for 0.5 h or 8 h until being sacrificed (Borg et al., 2011; Crowe et al., 2009). For the *in vivo* acute PEDF (50 μg) combined ISO (6 μg, Sigma, St. Louis, MO, USA) injection, mice ($n = 5$) were maintained for 8 h until being sacrificed. For PEDF antibody blockage in obese mice, mice ($n = 3$) that were fed the HFD for 16 weeks were injected intraperitoneally with IgG (Beyotime, China) or PEDF antibody (Genscript, China) for 7 days, and they were later sacrificed for adipose tissue and plasma collection after 8 h of fasting. All blood and tissue samples isolated from the mice were immediately stored on ice.

2.3. Cell culture

The 3T3-L1 cell line was purchased from the Cell Bank of China Science Academy (Shanghai, China). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Gaithersburg, MD, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, GIBCO, Gaithersburg, MD, USA), and incubated at 37 °C in a humidified incubator at 5% CO_2 until use. 3T3-L1 cells were grown to confluence and induced to differentiate as described previously (Borg et al., 2011). Briefly, 2 days post-confluence (defined as day 0), cells were incubated with differentiation medium containing 0.5 mmol/l isobutylmethylxanthine (IBMX), 1 μM dexamethasone (DEX), 10 μg/ml insulin and 10% FBS for 3 days (IBMX, DEX and insulin were obtained from Sigma, St. Louis, MO, USA). Then, cells were exposed to DMEM with 10 μg/ml insulin and 10% FBS for another 2 days, they were then switched to DMEM with 10% FBS. Maturation of adipocytes was confirmed by BODIPY493/503 (Molecular Probes, Eugene, OR, USA) staining of the lipid droplets that were visualized under an Axioskop microscope (Carl Zeiss, Oberkochen, Germany) at day 9. All cells were starved of serum for 10 h prior to the experiment.

2.4. Purification of recombinant PEDF and construction of mouse ATGL plasmid

Recombinant PEDF was expressed and purified as described previously (Xu et al., 2011). In brief, the pET30a(+)/PEDF construct was expressed by the BL21 (DE3) *Escherichia coli* strain (Novagen, Madison, WI, USA) and purified with Ni–NTA His-Bind resin (Novagen, Madison, WI, USA) using FPLC. Recombinant PEDF was confirmed by SDS–PAGE and western blot analysis. Plasmids