Physiology & Behavior 105 (2012) 777–783

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

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

Regulation of lipin1 by nutritional status, adiponectin, sex and pituitary function in rat white adipose tissue

C. Ruth González [a,b], Marta G. Novelle [a,b], Jorge E. Caminos [c], María J. Vázquez [a,b], Raul M. Luque [b,d], Miguel López [a,b], Ruben Nogueiras [a,b,*], Carlos Diéguez [a,b,*]

[a] Department of Physiology, School of Medicine-Instituto de Investigaciones Sanitarias (IDIS), University of Santiago de Compostela, Spain
[b] CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Santiago de Compostela 15782, Spain
[c] Department of Physiology and Genetic Institute, Faculty of Medicine, National University of Colombia, Bogotá, Colombia
[d] Department of Cell Biology, Physiology and Immunology, University of Cordoba, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), 14014 Córdoba, Spain

ARTICLE INFO

Article history:
Received 7 February 2011
Received in revised form 16 August 2011
Accepted 13 October 2011

Keywords:
Lipin1
Nutritional status
Pregnancy
Sex steroids
Pituitary alterations

ABSTRACT

Lipin1 is a member of the lipin protein family that plays an important role in the regulation of lipid metabolism. The endogenous role of lipin1 was demonstrated by the fact that mutations in lipin1 caused lipodystrophy and metabolic disorders. The aim of this study was to assess the influence of nutritional status, pregnancy, insulin-sensitizers and pituitary hormones on lipin1 mRNA levels in adipose tissue of rats. Lipin1 gene expression was induced in conditions of hypoleptinemia (fasting) and leptin resistance (high fat diet), whereas it was decreased by high circulating leptin levels (leptin administration, pregnancy) and in leptin-deficient mice. Lipin1 mRNA levels were also decreased in adiponectin-deficient mice. Lipin1 mRNA levels are influenced by age in female rats, with peak expression at 25th day of life and decreasing thereafter. Consistently, ovariectomy increased lipin1 expression indicating that estrogens modulate lipin1. Finally, lipin1 was also regulated by pituitary hormones, since its expression was modified by thyroid status and growth hormone deficiency.

Our observations indicate that: a) gWAT lipin1 mRNA levels are regulated by nutritional status, and leptin plays an important role in this regard, b) lipin1 is modulated by adiponectin, c) lipin1 is influenced by age and sex, and d) alterations in pituitary function modify lipin1 mRNA levels. To dissect the complicated interactions between key regulators of lipid metabolism like lipin1, may be important for the development of new therapies for the treatment and prevention of obesity and its associated disorders.

1. Introduction

White adipose tissue (WAT), in addition to the storage of lipids function, plays a crucial role in the maintenance of energy homeostasis. Alterations in the normal function of WAT are associated with the development of several diseases, such as insulin resistance, type II diabetes mellitus, and cardiovascular complications [1–3].

Lipodystrophy is a heterogeneous group of disorders characterized by loss of body fat, fatty liver, hypertriglyceridemia, and insulin resistance. In mice, using a positional cloning method [4], one of the genes responsible for fatty liver dystrophy, characterized by neonatal fatty liver and hypertriglyceridemia and neuropathy affecting peripheral nerve in adulthood, was isolated and designated lipin1. Lipin 1 is a Mg^{2+}-dependent phosphatidate phosphatase enzyme catalyzing the dephosphorylation of phosphatidic acid, yielding inorganic phosphate and diacylglycerol needed for the synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine [27,36–38]. Two mutant alleles of the lipin1 gene, fatty liver dystrophy 1 and 2 (fld1 and fld2), were associated with a markedly reduced adipose tissue mass [4]. Lipin1 is predominantly expressed in white and brown adipose tissue as well as in muscle [4]. Transgenic mice overexpressing lipin1 in either skeletal muscle or adipose tissue were also shown to have high rates of obesity [5]. Contrarily, lipin1 deficiency prevents the differentiation of human adipocyte precursor cells *in vitro* and causes lipodystrophy syndrome *in vivo* [6]. Lipodystrophy in the lipin1 knockout (KO) mice can be attributed to a combination of impaired lipid storage in adipose tissue and altered energy metabolism in muscle. Lipin1 in adipose

* Corresponding authors at: Department of Physiology, School of Medicine-Instituto de Investigaciones Sanitarias (IDIS), University of Santiago de Compostela and CIBER Fisiopatología de la Obesidad y la Nutrición (CIBERobn), S. Francisco s/n, 15782, Santiago de Compostela (A Coruña), Spain. Tel.: +34 981582658; fax: 34 981 582 642.
E-mail addresses: ruben.nogueiras@usc.es (R. Nogueiras), carlos.dieguez@usc.es (C. Diéguez).

0031-9384/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2011.10.016

tissue has an important role in the capacity to store fat in adipocyte. For instance, lipin1 deficiency impairs adipocyte differentiation and causes lipodystrophy in mice [4], whereas the overexpression of lipin1 in adipose tissue promotes obesity by enhancing lipogenic gene expression [5]. In skeletal muscle it is a key determinant of whole-body energy expenditure and fat expenditure [5]. Furthermore, the inability of lipin1 KO mice to store energy in adipose tissue leads to a compensatory increase in glycogen storage. The glycogen is then used during the fasting period and the mice rely upon hepatic fatty acid synthesis to provide fuel for peripheral tissues during the fed state [7].

In humans, lipin1 has been linked to lipodystrophy, while lipin1 polymorphisms have been associated with numerous metabolic traits, including insulin and glucose levels [8,9], resting metabolic rate [8], and systolic blood pressure [10,11]. However, lipin1 expression levels in adipose tissue are positively correlated with insulin sensitivity, and inversely correlated with inflammatory cytokine expression and intramyocellular lipid, a key marker of insulin resistance. Therefore, a detailed analysis of the regulation of lipin1 levels in different experimental settings is clearly warranted.

Despite recent advances in defining the intracellular function and gene regulation of lipin1, the molecular mechanisms mediating its actions and its physiological and pathophysiological roles on the adipose tissue homeostasis remain largely unknown. These are particularly relevant issues since it has been proposed that the increase/decrease of fat mass can be at the root of the metabolic consequences of either obesity or lipodystrophy. The aim of this study was to analyze the regulation of lipin1 in rodent WAT in different physiological (nutritional status, pregnancy, age, and sex) and pathophysiological settings (gonadectomy, thyroid status and growth hormone deficiency), all of which are known to be associated with energy homeostasis and alterations in insulin sensitivity.

2. Materials and methods

2.1. Animal models and strains

This study used several different rat models: male and female Sprague–Dawley rats (Bred in the Animalario General USC; Santiago de Compostela, Spain); male dwarf (HsdOla: dw-4) Lewis rats (150–175 g, 10–12 weeks old) (Harlam Ibérica; Barcelona, Spain); male Lewis rats age-matched with dwarf Lewis rats (250–300 g, 10–12 weeks old) (Harlam Ibérica, Spain), male leptin-deficient mice (ob/ob) (8 weeks old, purchased from Charles River, Barcelona, Spain) and male adioponectin-deficient mice [12]. Animals were housed under conditions of controlled illumination (12:12-h light/dark cycle), humidity, and temperature. Animals were fed with a standard diet and tap water *ad libitum*. 8 animals per group were used in each experimental protocol. Surgical procedures were performed under anesthesia by an intraperitoneal injection of ketamine/xylazine (ketamine 100 mg/kg body weight plus xylazine 15 mg/kg body weight as previously described [13]). The animals were sacrificed by decapitation in a room separate from other experimental animals in the afternoon (16:00–17:00 h). Gonadal WAT (gWAT) pad was then collected and frozen at −80 °C until mRNA analysis. All experimental procedures in this study were reviewed and approved by the Ethics Committee of the University of Santiago de Compostela in accordance with our institutional guidelines and the European Union normative for the care and use of experimental animals. Adiponectin −/− mice were sacrificed by decapitation in the morning and the experimental procedures with these animals were reviewed and approved by Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2.2. Experimental setting 1: effects of food deprivation and leptin on lipin1 mRNA levels

The effects of systemic leptin administration on gWAT lipin1 mRNA levels were studied in 10-week-old male wild-type Sprague–Dawley rats and ob/ob mice as described elsewhere [13,14]. Animals were assigned to one of the following groups: the first group was allowed free access to food; the second group was food-deprived for 48 h; and the third group was fasted for 48 h and treated with recombinant leptin (Sigma) at a dose of 0.5 μg/g of body weight every 6 h for 3 days (intraperitoneal injection).

2.3. Experimental setting 2: the influence of high-fat diet on lipin1 gene levels

It is well known that rodents on high-fat diets develop leptin resistance in the ARC [15]. 3-week-old male wild-type rats were either fed a high-fat diet (45% by energy) or a low-fat diet (10% by energy) (reference #: D12451 and D12450B respectively, Research Diets, NJ, US) for 12 weeks.

2.4. Experimental setting 3: effect of chronic food restriction on lipin1 mRNA levels

8-week-old male wild-type rats were randomly assigned to one of two dietary groups on day 1 as previously described [16,17]: rats were either fed *ad libitum* or food restricted to 30% of the amount of food consumed by *ad libitum* fed rats on the previous day. Rats were sacrificed after 8, 16, and 21 days on the restricted diet and gWAT samples collected. Tissues were frozen at −80 °C until processing. We used 8 animals per experimental group.

2.5. Experimental setting 4: lipin1 mRNA levels throughout gestation

Lipin1 mRNA levels were studied throughout gestation in gWAT of wild-type Sprague Dawley rats, according to the method described elsewhere [17,18]. Female rats were mated on the day of proestrus at approximately 10-weeks-old. The first day of pregnancy was documented by the presence of a vaginal plug with sperm after mating. gWAT was dissected from pregnant rats sacrificed on days 16, 19, and 21 of gestation.

2.6. Experimental setting 5: effect of adiponectin deficiency on lipin1 mRNA levels

Since adiponectin KO mice show important alterations in fatty acid metabolism, we measured the mRNA levels of lipin1 and several key lipogenic enzymes in the gWAT of adult (10 week-old) male mice lacking adiponectin.

2.7. Experimental setting 6: influence of age and sex on lipin1 mRNA levels

To analyze the effect of age and sex on gWAT lipin1 mRNA levels, male and female wild-type Sprague Dawley rats of the subsequent ages were studied: 15, 25, 45, 60 and 90 day-old [13,19].

2.8. Experimental setting 7: effects of gonadal hormones on lipin1 mRNA levels

In order to analyze the effect of gonadal hormones on gWAT lipin1 mRNA levels, adult male and female wild-type rats were bilaterally orchidectomized (ORX), ovariectomized (OVX), or sham operated as previously described [17,20]. Two weeks after surgery the different groups of rats were sacrificed.