Original article

Lipodystrophy and severe metabolic dysfunction in mice with adipose tissue-specific insulin receptor ablation

Guifen Qiang [1], Hyerim Whang Kong [1], Shanshan Xu [1], Hoai An Pham [2], Sebastian D. Parlee [2], Aaron A. Burr [2], Victoria Gil [1], Jingbo Pang [3], Amy Hughes [1], Xuejiang Gu [1,4], Giamila Fantuzzi [3], Ormond A. MacDougald [2], Chong Wee Liew [1,*]

ABSTRACT

Objective: Insulin signaling plays pivotal roles in the development and metabolism of many tissues and cell types. A previous study demonstrated that ablation of insulin receptor (IR) with aP2-Cre markedly reduced adipose tissues mass and protected mice from obesity. However, multiple studies have demonstrated widespread non-adipocyte recombination of floxed alleles in aP2-Cre mice. These findings underscore the need to re-evaluate the role of IR in adipocyte and systemic metabolism with a more adipose tissue-specific Cre mouse line.
Methods: We generated and phenotyped a new adipose tissue-specific IR mouse model using the adipose tissue-specific Adipoq-Cre line.
Results: Here we show that the Adipoq-Cre-mediated IR KO in mice leads to lipodystrophy and metabolic dysfunction, which is in stark contrast to the previous study. In contrast to white adipocytes, absence of insulin signaling does not affect development of marrow and brown adipocytes, but instead is required for lipid accumulation particularly for the marrow adipocytes. Lipodystrophic IR KO mice have profound insulin resistance, hyperglycemia, organomegaly, and impaired adipokine secretion.
Conclusions: Our results demonstrate differential roles for insulin signaling for white, brown, and marrow adipocyte development and metabolic regulation.

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Keywords Insulin signaling; White adipose tissue; Lipodystrophy; Marrow adipose tissue; Brown adipose tissue

1. INTRODUCTION

Adipose tissue is a complex organ, playing an active role in whole-body energy and metabolic homeostasis. In mammals, adipose tissue is composed of at least two functionally distinct types, namely brown (BAT) and white (WAT) adipose tissue, which are characterized by their opposing metabolic properties [1,2]. BAT is specialized for energy expenditure and adaptive thermogenesis in response to cold exposure or diet, whereas WAT is the primary energy reservoir and a major source of metabolic fuel in mammals in response to energetic demands. To regulate metabolism systemically, adipose tissues synthesize and secrete a wide variety of regulatory hormones called adipokines, including leptin and adiponectin [3–6]. Adipose tissue dysfunction has therefore been shown to play a critical role in the development of metabolic and cardiovascular diseases [7–9], with both an excess (i.e. obesity) and insufficiency (i.e. lipodystrophy) of adipose tissue leading to insulin resistance, dyslipidemia, and hepatic steatosis [10–12].

Insulin is an important regulator of intermediary and lipid metabolism and has both direct and indirect effects on most tissues in the body. Whole-body and tissue-specific disruption studies have demonstrated the role of insulin signaling in development and metabolism. Mice with global knockout (KO) of the insulin receptor (IR) die 4–5 days after birth due to severe ketosis [13]. However, the use of the Cre/LoxP system to perform tissue-specific KO has permitted further investigation in a number of tissues, including liver, pancreatic β-cells, brain, and muscle [14–17].
To explore IR roles in adipose tissues, several lines of Cre recombinase have been developed. The mouse adipocyte protein 2 promoter-driven Cre line (aP2-Cre) has been used to examine the 'adipose-specific' functions of IR in transgenic mice [18]. Previous studies demonstrated that fat-specific IR KO (FIRKO) mice have markedly reduced white adipose mass and whole-body triglyceride content. The FIRKO mice are protected from gold thioglucose-induced and age-related obesity, as well as the associated glucose intolerance. In addition to adipose tissues, however, aP2-directed Cre activities are also detectable in other tissues and cell types, including brain, endothelial cells,

[1]Department of Physiology & Biophysics, University of Illinois at Chicago, Chicago, IL, USA [2]Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI, USA [3]Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, IL, USA

[4] Current address: Endocrinology and Metabolism Department, 1st Affiliated Hospital of Wenzhou Medical University, Wenzhou, 325000, Zhejiang, PR China.

*Corresponding author. Department of Physiology & Biophysics, College of Medicine, University of Illinois at Chicago, 835 S Wolcott Ave, M/C 901, MSB, E-202, Chicago, IL 60612, USA. Tel.: +1 312 413 1086; fax: +1 312 996 1414. E-mail: cwliew@uic.edu (C.W. Liew).

Received March 24, 2016 • Revision received April 26, 2016 • Accepted May 6, 2016 • Available online 14 May 2016

http://dx.doi.org/10.1016/j.molmet.2016.05.005

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macrophages, adipocyte precursors, and embryonic tissues [18–22]. These studies call into question the specificity of the previous findings with IR knockout [23,24] and underscore the need to re-evaluate the role of IR in a more adipose tissue-specific Cre mouse line. Multiple groups have demonstrated that the adiponectin promoter-driven Cre (Adipoq-Cre) line [25] is highly fat-specific and effective in inducing recombination [18,26]. Here, using the Adipoq-Cre mouse, we generated a more specific IR fat KO (IR^{FKO}) mouse to re-assess the role of insulin signaling in adipocyte development and biology. In contrast to previous studies [24], Adipoq-Cre-mediated IR KO led to severe lipodystrophy rather than simply reduced fat mass. Furthermore, the lipodystrophic IR^{FKO} mice have profound insulin resistance, hyperglycemia, organomegaly (liver, heart, pancreas, kidney, spleen), and impaired adipokine secretion. Our model reveals that absence of insulin signaling in adipose tissues leads to a dramatic decrease in WAT depots weight due to reduced size and number of adipocytes. Our results also show that absence of insulin signaling significantly affects lipid accumulation in marrow and brown adipocytes but not adipocyte development in these locations. Taken together, our results establish that insulin signaling in adipocytes is required for adipocyte development and lipid accumulation in adipocytes, as well as for regulation of systemic metabolic homeostasis.

2. MATERIAL AND METHODS

2.1. Animals

Adipoq-Cre and $IR^{fl/fl}$ mice were obtained from the Jackson Laboratory. Both lines are on a C57BL/6 background. $IR^{fl/fl}$ littermates were used as control for all experiments. Mice were housed in environmentally controlled conditions with a 12-h light/dark cycle and had free access to standard rodent pellet food and water. The animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of University of Illinois at Chicago. Animal care was given in accordance with institutional guidelines.

2.2. Metabolic parameters

Plasma insulin was measured with an ELISA kit (RayBiotech). Non-esterified fatty acids (NEFA), triglyceride (TG), and cholesterol concentration in serum were measured with NEFA-C, Triglyceride E tests (Wako) and cholesterol liquicolor kits (Stanbio), respectively. Serum adiponectin and leptin levels were measured with ELISA kits from R&D Systems.

2.3. Physiological studies

Blood glucose was monitored with an automated glucose monitor (Glucometer Elite, Bayer). Glucose tolerance tests and insulin tolerance tests were performed 16 h after fasting, as described previously [15]. Mice were euthanized and tissues were rapidly dissected, weighed, and processed for downstream analyses as described previously [15]. Fat and lean masses were measured by DEXA (Norland Stratec) scanning.

2.4. RNA extraction and real-time PCR

Total RNA was isolated from tissues and cells with the use of Trizol reagent (Invitrogen) and Direct-zol kit (Zymo). cDNA was prepared from 1 μg of total RNA using the High Capacity cDNA Reverse Transcription Kit (Invitrogen) with random hexamer primers, according to the manufacturer's instructions. The resulting cDNA was diluted 5-fold, and a 1.5 μl aliquot was used in a 6 μl PCR reaction (SYBR Green, Bio-Rad) containing primers at a concentration of 300 nM each. PCR reactions were run in triplicate and quantitated using the Applied Biosystems ViiA™7 Real-Time PCR system. Results were normalized to *TATA box binding protein* (*TBP*) expression and expressed as arbitrary units or fold change.

2.5. Staining by osmium tetroxide

Mouse tibiae were stained using osmium tetroxide to analyze marrow lipid by microcomputed tomography (μCT), as previously described [27–29]. Tibia were fixed for 24 h in 10% neutral-buffered formalin (VWR), washed with water, and subsequently decalcified using 14% EDTA, pH 7.4, for at least 14 days or until bones were mechanically malleable. Bones were washed twice more with water and stored in Sorensen's phosphate buffer (pH 7.4) until analyzed. Due to the severe toxicity of osmium tetroxide, all remaining steps were performed using extreme caution and in a fume hood. Osmium tetroxide (Electron Microscopy Services) was added to each tibia as a 1% final solution for 48 h at room temperature. The osmium solution was carefully removed to a waste bottle containing corn or olive oil, and all tips were washed with oil to deactivate the compound. Tibiae were washed twice in fresh Sorensen's phosphate buffer for 2 h, and once more in Sorensen's overnight. Bones were moved to a fresh microfuge tube containing Sorensen's buffer, and used for CT analysis. All wash waste was disposed of as indicated above.

2.6. μCT bone morphology analysis

μCT analysis was performed as previously described [27,28]. Briefly, bones were embedded in 1% agarose and inserted into a tube 19 mm in diameter. The bones were scanned using a μCT system (μCT 100 Scanco Medical). μCT scan settings include: 12 μm voxel size, medium resolution, 70 kVp, 114 μA, 0.5 mm AL filter, and 500 ms integration time. Marrow density measurements were calibrated to the hydroxyapatite phantom of the manufacturer. Measurements were analyzed using manufacturer's evaluation software using a threshold of 400 for MAT.

2.7. Histology

Tissues were fixed in 10% (wt/vol) buffered formalin, and paraffin-embedded sections were subjected to H&E staining. For MAT, samples were fixed in 10% neutral-buffered formalin, decalcified in 14% EDTA (pH 7.4) for at least 14 days. After paraffin embedding, bones were sectioned and stained with hematoxylin and eosin. Adipocyte size distribution was determined using MetaMorph Image Analysis software as previously described [30,31].

2.8. Body temperature and cold exposure

7-week-old mice were exposed to an ambient temperature of 4 °C in a cold room until their core body temperature dropped to around 30 °C. Body temperatures were measured at 30 min intervals using a RET-3 rectal probe for mice (Physitemp).

2.9. Western blot

Total cell or tissue lysates (40 μg) were subjected to SDS–PAGE and blotting was performed as described with anti-UCP1 (Abcam) or anti-β-actin (Proteintech) antibodies [32]. Multiple exposures were used to ascertain signal linearity.

2.10. Statistical analyses

All data are presented as the mean ± S.E.M. and were analyzed by unpaired two-tailed Student's *t* test or analysis of variance, as appropriate. $P < 0.05$ was considered significant. MAT data are presented as mean ± SD and were analyzed statistically by ANOVA with Tukey/Sudak posthoc analysis using GraphPad Prism.

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