Original Article

MOLECULAR METABOLISM

Adipocyte glucocorticoid receptor is important in lipolysis and insulin resistance due to exogenous steroids, but not insulin resistance caused by high fat feeding

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ABSTRACT

Objective: The critical role of adipose tissue in energy and nutrient homeostasis is influenced by many external factors, including overnutrition, inflammation, and exogenous hormones. Prior studies have suggested that glucocorticoids (GCs) in particular are major drivers of physiological and pathophysiological changes in adipocytes. In order to determine whether these effects directly require the glucocorticoid receptor (GR) within adipocytes, we generated adipocyte-specific GR knockout (AGRKO) mice.

Methods: AGRKO and control mice were fed chow or high fat diet (HFD) for 14 weeks. Alternatively, AGRKO and control mice were injected with dexamethasone for two months. Glucose tolerance, insulin sensitivity, adiposity, lipolysis, thermogenesis, and insulin signaling were assessed.

Results: We find that obesity, insulin resistance, and dysglycemia associated with high fat feeding do not require an intact GR in the adipocyte. However, exogenous dexamethasone (Dex) promotes metabolic dysfunction in mice, and this effect is reduced in mice lacking GR in adipocytes. The ability of Dex to promote "whitening" of brown fat is also reduced in these animals. We also show that GR is required for β-adrenergic and cold stimulation-mediated lipolysis via expression of the key lipolytic enzyme ATGL.

Conclusions: Our data suggest that the GR plays a role in normal adipose physiology via effects on lipolysis and mediates at least some of the adverse effects of exogenous steroids on metabolic function. The data also indicate that intra-adipocyte GR plays less of a role than previously believed in the local and systemic pathology associated with overnutrition.

Keywords Glucocorticoid receptor; Adipose tissue; Insulin resistance; Lipolysis; Dexamethasone

1. INTRODUCTION

Adipose tissue plays an important role in energy homeostasis, serving not only as the primary depot for caloric storage but also as an integrator of nutrient, hormonal, and immune signaling [1]. Under normal conditions, adipose tissue regulates processes as varied as appetite, thermogenesis, the fasting–feeding transition, blood pressure, and others. In overnutrition, adipose tissue becomes dysfunctional, developing progressive insulin resistance within the adipocyte compartment itself and ultimately contributing to systemic alterations in insulin sensitivity and lipid handling.

Among the hormonal factors that participate in the control of adipose behavior, glucocorticoids (GCs) are among the best studied. GCs (mainly cortisol in man and corticosterone in rodents) are produced by the adrenal cortex under the control of the hypothalamus and pituitary gland. GCs have been implicated in multiple aspects of adipose tissue biology, including fat cell differentiation [2–4], lipogenesis and lipolysis [5], and thermogenesis [6–10]. Exposure to high levels of endogenous or exogenous GCs causes profound adipose remodeling, including central obesity and peripheral wasting, as well as severe insulin resistance and hyperglycemia [11]. In mice, administration of GCs causes metabolic dysfunction that is highly reminiscent of that seen in human studies and transgenic overexpression of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) in fat, which increases active GC levels and leads to increased adipose tissue mass, insulin resistance, and diabetes [12].

In our prior work, we have focused on the mechanisms by which cellular insulin resistance develops in adipocytes. We noted that insulin resistance is a common phenotype associated with both pro-inflammatory cytokines and anti-inflammatory GCs like dexamethasone (Dex), and we utilized genomic and epigenomic approaches to identify common pathogenic pathways [13,14]. Interestingly, the

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Abbreviations

GCs	Glucocorticoids
GR	Glucocorticoid receptor
MR	Mineralocorticoid receptor
AGRKO	Adipocyte-specific GR knock out
Flox	$GR^{flox/flox}$ mice
HFD	High fat diet
Dex	Dexamethasone
11β-HSD1	11β-Hydroxysteroid dehydrogenase 1
eWAT	Epididymal white adipose tissue
iWAT	Inguinal white adipose tissue
NEFA	Nonesterified fatty acids
TG	Triglycerides
TRAP	Translating ribosome affinity purification
MRI	Magnetic resonance imaging
Gas	Gastrocnemius
Sol	Soleus

glucocorticoid receptor (GR, encoded by *Nr3c1*) was implicated in these studies as a driver of insulin resistance in the presence of either Dex or TNF-α.

Here we explore three questions: 1) To what extent does GR within adipocytes participate in the development of insulin resistance in living animals, 2) do exogenous GCs cause metabolic dysfunction by acting through the GR in adipocytes, and 3) what other adipocyte functions are regulated by intrinsic GR? To address these questions, we deleted the GR selectively in adipocytes using the cre-loxP system in mice. We demonstrate that adipocyte GR participates in lipolysis but, surprisingly, does not contribute to altered glucose homeostasis or insulin resistance in the setting of diet-induced obesity. Administration of Dex, however, causes insulin resistance that depends upon the presence of GR in adipocytes.

2. METHODS

2.1. Animal experiments

To generate AGRKO mice, we crossed Adiponectin-Cre mice (Jackson Laboratory, 010803) [15] with GR floxed mice (Jackson Laboratory, 021021). For high fat feeding studies, 6-week old littermate Flox ($GR^{flox/flox}$) and AGRKO mice were fed 58% high fat diet (12331i, Research Diets) for 14 weeks. For studies using dexamethasone (Dex), 16-week old littermate Flox and AGRKO mice were administered saline or dexamethasone sodium phosphate (3 mg/kg body weight; Santa Cruz Biotechnology) intraperitoneally every other day for 2 months. Body weight was measured weekly. Mice were subjected to magnetic resonance imaging (MRI) (Echo Medical Systems) to examine body compensation. Tissues were harvested, frozen in liquid nitrogen, and stored at −80 °C until used. For cold exposure experiments, mice were placed at 4 °C for up to 6 h. Body temperature was measured using a rectal probe (Yellow Spring Instruments). All experiments were approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

2.2. Glucose and insulin tolerance test

For glucose tolerance tests, mice were fasted for 12 h and injected with glucose intraperitoneally (2 g/kg body weight for HFD studies; 1 g/kg body weight for Dex studies). Blood samples were collected at 0, 15, 30, 60, 90, and 120 min. For insulin tolerance tests, mice were fasted for 5 h and injected with insulin (1.5 U/kg body weight for HFD studies; 1 U/kg body weight for Dex studies). Blood samples were collected at 0, 15, 30, 60, 90, and 120 min. Glucose levels were measured with a handheld glucometer (One Touch Ultra Mini).

2.3. RNA isolation and quantitative PCR

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen) following the manufacturer's instructions. Total RNA (500 ng) was converted into cDNA, and quantitative PCR (qPCR) was performed using SYBR Green qPCR Master Mix (Applied Biosystems) using a 7900HT Fast Real Time PCR system. The relative abundance of mRNA was standardized with *36B4* mRNA as the invariant control. Primer sequences are provided in Supplemental Table 1.

2.4. Protein extraction and western blotting

Protein was extracted from tissues in RIPA buffer (Boston BioProducts) supplemented with complete protease inhibitor cocktail (Roche). Fat pads were fractionated into adipocytes and non-adipocytes as described [16]. Lysates were separated by 4–15% gradient SDS-PAGE and transferred to PVDF membrane (Millipore). The following antibodies were used: Akt (#9272), p-Akt (S473) (#9271), p70S6K (#2708), p-p70S6K (#9205), β-actin (#4970), and α/β-tubulin (#2148), all from Cell Signaling Technology. GR (M-20) was from Santa Cruz. *UCP-1* (ab10983), total OXPHOS (ab110143), and Tomm20 (ab56783) were from Abcam. All blots were quantified using ImageJ software (National Institutes of Health, Bethesda, MD).

2.5. Histology

Collected fat tissues were fixed in 4% formaldehyde for 48 h at 4 °C and washed with PBS. Paraffin embedded tissues were stained with H&E, and images were acquired using a Zeiss-Axio Imager A1 microscope. Adipocyte size was quantified using ImageJ software (NIH).

2.6. Plasma parameters

Blood samples were collected from fasted mice in EDTA-coated blood collection tubes and plasma insulin levels were measured by ELISA kit according to the manufacturer's instructions (Crystal Chem). Plasma levels of triglycerides and nonesterified fatty acids (NEFA) were determined using kits from Thermo Scientific and Wako Diagnostics, respectively.

2.7. Lipolysis assay

For *in vivo* studies, mice were fasted for 4 h and injected with isoproterenol (10 mg/kg body weight). Blood was collected from the tail vein before and 20 min after injection. Glycerol was determined using a kit from Sigma–Aldrich, and NEFA content was measured as above. For *ex vivo* studies, epididymal fat pads were surgically removed from male mice and washed with ice-cold PBS. Excised adipose tissue pads (10–20 mg) were incubated in the presence or absence of 1 mM isoproterenol with DMEM for 0–2 h at 37 °C with gentle shaking.

2.8. Insulin signaling

Mice were fasted overnight, followed by IP insulin injection (10 U/kg body weight). Ten minutes later, tissues were collected and stored at −80 °C until use. Tissue samples were homogenized in RIPA buffer containing protease inhibitor (Roche) and phosphatase inhibitor (Sigma–Aldrich) and subjected to western blotting.

2.9. Statistical analysis

All data are presented as mean ± SEM. Unpaired two-tailed student's *t*-test and two-way ANOVA were used. p Values < 0.05 were considered statistically significant.

