Effect of Obesity on Resistin and Adenylyl Cyclase-Associated Protein (CAP1) Expression in White Adipose Tissue

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ABSTRACT

Introduction: Resistin is a cytokine which is produced by the white adipose tissue (WAT) and contributes to insulin resistance and type 2 diabetes. Recently, adenylyl cyclase-associated protein 1 (CAP1) was identified as a receptor for resistin, however, little information is available about CAP1 relationship with resistin.

Materials and Methods: 6-weeks old male C57Bl/6J mice were fed regular diet (RD) or high-fat diet (HFD) for 9 weeks. After overnight fast, body measurements were taken and mice were euthanized. Various WAT depots samples were collected. WAT tissue samples were subjected to hematoxylin & eosin, resistin or CAP1 staining. Blood glucose levels were measured by capillary glucose meter. Plasma levels of insulin, tumor necrosis factor alpha (TNFa), interleukin-6 (IL-6), leptin, monocyte chemotactic protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) were measured by MILLIPLEX assay. Plasma CAP1 levels were measured by ELISA. Insulin sensitivity and steady state β cell function were evaluated by quantitative insulin sensitivity check index (QUICKI) and homeostatic model assessment 2 (HOMA2) index.

Results: HFD regimen resulted in 40% increase in animal body weight. HFD group of mice displayed significantly higher WAT accumulation with approximately 6-fold higher epididymal WAT mass compared to the control RD group. Additionally, HFD-fed animals had higher liver mass and prominent hepatocyte lipid accumulation. HFD mice were characterized by elevated plasma fasting glucose and insulin levels and both, QUICKI and HOMA-IR indexes demonstrated impaired insulin sensitivity. Further, HFD-fed mice showed signs of chronic inflammation manifested by higher incidence of crown-like structures (CLS) in WAT and upregulated circulating levels of proinflammatory cytokines (TNFa, IL-6, leptin, MCP-1, and PAI-1). Immunohistochemical staining of WAT slices revealed strong resistin and CAP1 expression in CLS.

Conclusions: Both, resistin and CAP1 proteins are highly expressed in mouse WAT. The adipose tissue expression of these proteins is concentrated in the CLS.

Additional studies are in progress.

Conflicts of interest disclosure: Authors declare no conflict of interest.

INTRODUCTION

Resistin is a newly discovered cytokine produced mainly by the white adipose tissue (WAT) and to a lesser extend by brown adipose tissue (BAT), hypothalamus, pituitary, adrenal glands and other organs.[1, 2, 3] Multiple clinical and *in vivo* studies involving both genetic or diet-induced obesity (DIO) animal models, found that serum resistin levels correlate with WAT mass reaching highest levels in states of obesity,[1, 4-8] and that weight loss is accompanied by a decrease in serum resistin levels.[9] Additionally, serum resistin levels correlate with markers of inflammation (soluble TNFα receptor-2 (TNFR-2), IL-6 and others)[10,11] and are indicative of type 2 *diabetes mellitus* (T2DM)[12] Research found that overexpression of resistin in metabolically healthy mice led to insulin resistance and dysregulated lipid metabolism with increased accumulation of triglycerides and cholesterol[13,14]. Conversely, mice lacking resistin show an improved glucose tolerance compared in both, wild-type and obese models.

Adenylyl cyclase-associated protein 1 (CAP1) was recently identified as a receptor for resistin.[15] Both, CAP1 mRNA and protein were found to be expressed in WAT[16] and to be secreted in the urine of patients with systemic autoimmune diseases.[17] Recent studies demonstrated that CAP1 mediates inflammatory actions of human monocytes.[15]

Taken together, these data strongly support the hypothesis that resistin and CAP1 are the major mediators connecting abdominal obesity with the development of insulin resistance and T2DM, and the insulin-resistance actions of resistin might be modulated by the CAP1.

MATERIALS & METHODS

Animals

In vivo experiment was performed in accordance with the National Institutes of Health (NIH) guidelines under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of The Feinstein Institute for Medical Research in Manhasset, NY.

Study design is presented on Figure 1. 6 weeks old C57Bl/6J mice (Jackson Laboratory, Bar Harbor, ME) were fed with either regular diet (RD) or 60% high-fat diet (HFD) for 9 weeks. Before sacrifice, the animals were overnight (18-20 hrs.) fasted and blood glucose levels were measured by using OneTouch Verio Flex™ System (Life Scan, Inc., Wayne, PA). Immediately after sacrifice, terminal blood was withdrawn by heart puncture. Animals were formaldehyde perfused and livers and various WAT samples were collected and stored in formaldehyde.

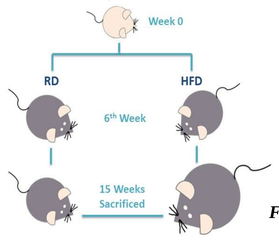

Figure 1. Study design.

Assessment of insulin sensitivity

Insulin sensitivity was assessed by quantitative insulin sensitivity check index (QUICKI) and homeostasis model assessment (HOMA) index.

Plasma adipokine levels measurements

Plasma levels of IL-6, insulin, leptin, MCP-1, PAI-1 (total), and TNFα were measured by using MILLIPLEX Map Mouse Adipokine Magnetic Bead Panel, Endocrine Multiplex Assay (EMD Millipore, Mahopac, NY).

Hematoxylin & eosin staining and Immunohistochemistry

Formaldehyde-stored tissue samples were transferred in 1x PBS and sent to the Histopathology Core facility at the New York University School of Medicine in New York, NY for paraffin embedding, tissue slicing and hematoxylin & eosin (H&E) staining.

For the immunohistochemistry, the formalin-fixed, paraffin-embedded tissue slices were de-paraffinized using xylene, dehydrated in decreasing concentrations of ethanol, and re-hydrated by using Ultra-Sensitive ABC Peroxidase Staining kit (Thermo Fisher Scientific, Hanover Park, IL). Antigens were retrieved by using microwave heating (20 min. in 0.01M citrate buffer, pH 6.0) after inhibition of endogenous peroxidase activity (1% hydrogen peroxidase for 30 min.). Slides were incubated with rabbit polyclonal anti-resistin and rabbit monoclonal anti-CAP1 (Abcam, Cambridge, MA) primary antibodies overnight at 4 °C, washed with PBS and further incubated with biotinylated anti-rabbit and anti-goat IgG secondary antibodies for 1 hr. at room temperature. The slides were then incubated with streptavidin-biotin-peroxidase complex for 30 min. at room temperature followed by another wash with PBS. A solution of diaminobenzidine (DAB) (Sigma-Aldrich, Saint Louis, MO) (1 mg/ml) was applied for 4 min. as a chromogen.

The stained slides were observed and analyzed by using EVOS FL Auto microscope (Life Technologies Corp., Carlsbad, CA).

RESULTS

Utilization of C57Bl6/J DIO mouse model

6-weeks old male mice were fed with regular (RD) or 60% high-fat (HFD) diet for 9 weeks. RD and HFD mice (Fig. 2A) were compared on body weight (Fig. 2B) and eWAT and ingWAT depots were individually weighed (Fig. 2C). Livers of HFD mice were significantly heavier (Figure 2D) and more pale in color (Fig. 2E). H&E staining of liver sections (Fig. 2F) demonstrated higher accumulation of lipids in hepatocytes (Fig. 2G).

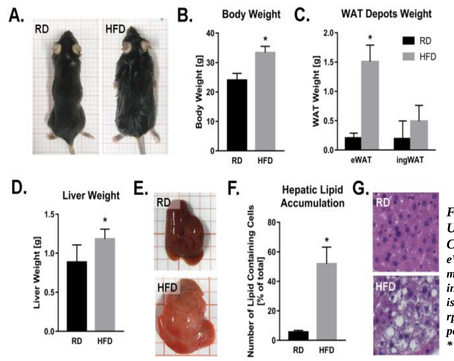

Figure 2. Utilization of C57Bl6/J DIO mouse model. *eWAT—epididymal WAT, mWAT—mesenteric WAT, ingWAT—inguinal WAT, isWAT—interscapular WAT, rpWAT—retroperitoneal WAT, pcWAT—pericardial WAT.* **** p < 0.05.***

DIO affect on adipocyte size in C57Bl/6J mice

Epididymal (eWAT), mesenteric (mWAT), inguinal (ingWAT), retroperitoneal (rpWAT), and pericardial (pcWAT) WAT were individually dissected, paraffin embedded and subjected to microscope sectioning. Hematoxylin and eosin (H&E) staining of these tissues demonstrated that 9-weeks of 60% HFD results in significant increase in adipocyte size (Fig. 3A and B). Additionally, the mice on HFD had significantly higher deposit of eWAT compared to their RD counterparts (Fig. 3C).

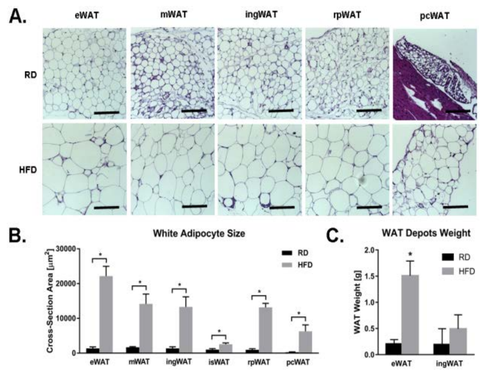

Figure 3. Comparisons of adipocyte size between mice on RD and HFD.

Induction of insulin resistance in DIO C57Bl/6J mice

Fasting plasma glucose and insulin (Fig. 4A) were measured.

Insulin resistance was evaluated by quantitative insulin sensitivity check index (QUICKI) (Fig. 4B) and homeostasis model assessment (HOMA) index (Fig. 4C). Results demonstrated that DIO C57Bl/6J mice had impaired insulin sensitivity.

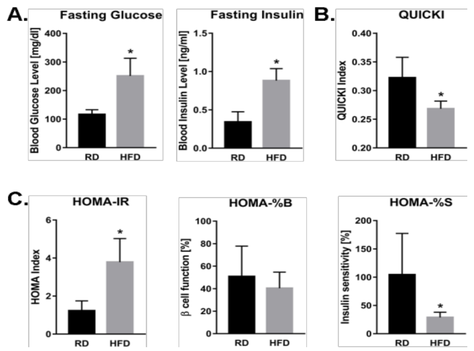

Figure 4. Induction of insulin resistance in DIO C57Bl/6J mice. *IR—insulin resistance, %B—percentage of steady state b-cell function, %S—insulin sensitivity.* **** p < 0.05.***

Inflammatory profile of DIO C57Bl/6J mice

HFD-fed mice demonstrated higher infiltration of WAT macrophages and presence of crown-like structures (CLS) (Fig. 5A and B). Levels of pro-inflammatory cytokines (TNFα, IL-6, leptin, MCP-1, and PAI-1) were measured by using MILLIPLEX analysis.

Data revealed that plasma levels of all examined cytokines were elevated in the HFD group of animals compared to the RD controls (Fig. 5C).

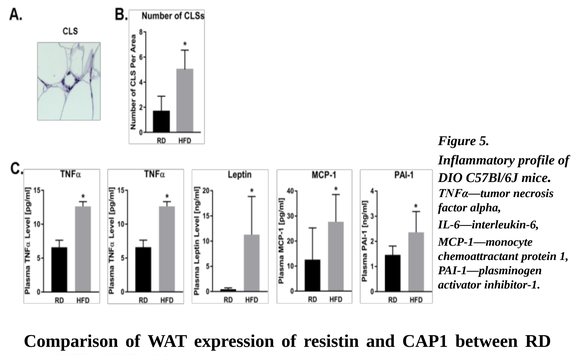

Figure 5. Inflammatory profile of DIO C57Bl/6J mice. *TNFα—tumor necrosis factor alpha, IL-6—interleukin-6, MCP-1—monocyte chemoattractant protein 1, PAI-1—plasminogen activator inhibitor-1.*

Comparison of WAT expression of resistin and CAP1 between RD and HFD C57Bl/6J mice

eWAT, ingWAT, and mWAT were subjected to immunohistochemistry to examine the tissue expression of resistin and CAP1. The immunohistochemical staining revealed that both resistin (Fig. 6A) and CAP1 (Fig. 6B) were highly expressed in WAT. The expression of resistin and CAP1 coincided in the CLS areas highly populated with adipose tissue macrophages (ATM).

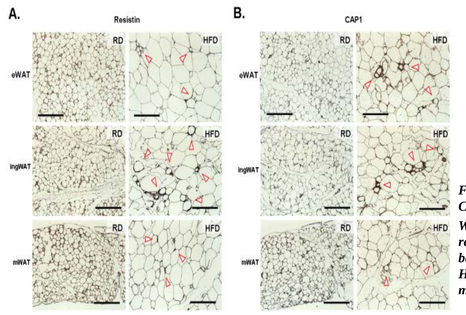

Figure 6. Comparison of WAT expression of resistin and CAP1 between RD and HFD-fed C57Bl/6J mice.

CONCLUSIONS

Obesity induces the formation of CLS in WAT, where both resistin and CAP1 are highly expressed.

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