Effect of High-Fat Diet on Cofilin and MMP9 Protein Expression in Diet-Induced Obesity Mouse Model

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ABSTRACT

Introduction: Resistin is a proinflammatory cytokine produced by the white adipose tissue (WAT) that is a major contributing factor for the development of insulin resistance. Recently, adenylyl cyclase-associated protein 1 (CAP1) was found to serve as a receptor for resistin. CAP1 directly regulates cofilin 1 protein function and thus G-actin fibers reorganization, which additionally affect cellular microtubule formation.

Materials and Methods: 6-weeks old male C57Bl/6J mice were fed regular diet (RD) or high-fat diet (HFD) for 9 weeks. Blood and various WAT depots samples were collected. The effect of HFD on WAT mRNA expression of resistin, CAP1, cofilin 1 and MMP9 was examined by qRT-PCR analyses. WAT protein expression of resistin, cofilin 1 and α-tubulin was examined by Western blot analyses. Plasma levels of resistin and CAP1 were measured by MILLIPLEX assay or ELISA.

Results: HFD mice showed significantly elevated basal resistin mRNA levels in both, the epididymal (e) and interscapular (is) WAT. CAP1 mRNA expression was upregulated in the eWAT, but not in the isWAT. HFD regimen induced cofilin 1 and suppressed MMP9 mRNA levels in isWAT. Additionally, HFD group of mice showed higher expression of resistin and α-tubulin proteins in eWAT and scWAT, but the levels of cofilin 1 protein were not significantly different between the two groups. Both, resistin and CAP1 were expressed in the blood of all mice, as the plasma resistin levels were upregulated in the HFD group. In the HFD group of mice, there was a significant inverse correlation between the plasma levels of resistin and CAP1.

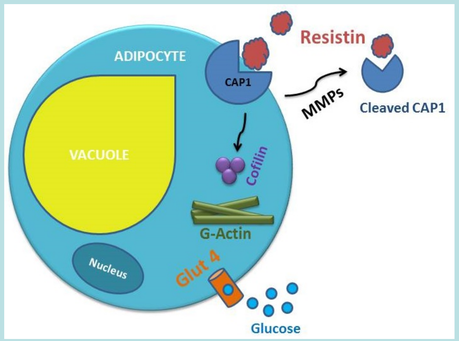
Conclusions: Taken together these data suggest that resistin may induce CAP1 signaling thus interfering with cytoskeletal proteins reorganization affecting in such a way glucose mechanism. Additional studies are in progress.

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

INTRODUCTION

Resistin is a proinflammatory adipokine produced mainly by the white adipose tissue (WAT)[1, 2, 3]. Resistin production is induced by obesity and is believed to play a major role in insulin resistance [1, 4-8, 9]. Recently, it was found that resistin can bind to adenylyl cyclase-associated protein 1 (CAP1) [10], but whether this could be a mechanism for induction of insulin resistance is still unknown. CAP1 directly regulates cofilin 1 protein function, which is involved in the G-actin fibers reorganization process by recycling the G-actin monomers[11]. There is a solid evidence that actin fibers directly interact with microtubules through specific bifunctional proteins or multiprotein complexes. Importantly, actin fibers and microtubule cytoskeleton reorganization plays a major role in variety of cellular processes including vesicle transferring[12].

HYPOTHESIS



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 described in details in Reference [1]. Briefly, 3 weeks old C57Bl/6J mice (Jackson Laoratory, Bar Harbor, ME) were fed with either regular diet (RD) or 60% high-fat diet (HFD) for 9 weeks. Mice were sacrificed and different WAT depots (epididymal [eWAT], interscapular [isWAT], subcutaneous [scWAT]) and blood samples were collected.

Quantitative RT-PCR analysis

RNA from WAT samples was extracted by using TRIzol®/chloroform/iso-propanol method, normalized to 1 µg/ml and converted to cDNA by using qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). Quantitative PCR analyses were performed by using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Hanover Park, IL) and QuantStudio 3 thermocycler (Life Technologies Corp., Carlsbad, CA). The primer sequences used for the PCR reactions are listed in Table 1. Relative mRNA expression levels were calculated by using $\Delta\Delta Ct$ method.

Table 1. List of primers.

Gene	Forward Primer [5'-3']	Reverse Primer [5'-3']
Resistin	TTCCTTGTCCCTGAACTGCT	TGCTGTCCAGTCTATCCTTG
CAP1	CCCAGCTACCTGCCTTCA	AGGCCTCAGGTAATGGGC
Cofilin 1	TCTGTCTCCCTTTCGTTTCC	TTGAACACCTTGATGACACCAT
MMP9	CCAACTATGACCAGGATAAAC	TTCTTGTCAGTGTCGAAGTTC
18s	AACCTGGTTGATCCTGCCAGT	GGCACCAGACTTGCCCTC

Western blot analysis

Protein from WAT samples was extracted by using Pierce™ RIPA Buffer (Thermo Fisher Scientific, Hanover Park, IL). Protein concentration was quantified by using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Hanover Park, IL), BioTek® plate reader and Gen5™ data analysis software (BioTek®, Winooski, VT). Protein extracts were normalized, separated by PAGE gel electrophoresis and transferred onto PVDF membranes. Membranes were blotted with Resistin (C-10) (Santa Cruz Biotechnology, Santa Cruz, CA), Cofilin 1 (Santa Cruz Biotechnology, Santa Cruz, CA), α-Tubulin (DM1A) Mouse mAb (Cell Signaling Technology, Danvers, MA), GAPDH (14C10) Rabbit mAb (Cell Signaling Technology, Danvers, MA) primary and Pierce™ Goat Anti-Mouse or Anti-Rabbit secondary antibodies (Thermo Fisher Scientific, Rockford, IL). Membranes were visualized by using SuperSignal West Pico Chemiluminescent Substrate and MyECL Imager (Thermo Fisher Scientific, Rockford, IL).

Statistical analysis

Statistical analysis were performed by unpaired *t*-test. Data were represented as mean values ± SEM. Data were accepted as statistically significant when $p < 0.05$.

RESULTS

Effect of HFD on resistin and CAP1 gene expression in C57Bl/6J mice

We measured resistin and CAP1 mRNA levels in eWAT and isWAT from mice from the RD and HFD groups. In both WAT depots, HFD was associated with significantly elevated resistin mRNA levels (Fig. 1A and B). Similarly to resistin, the levels of CAP1 mRNA were significantly upregulated in the eWAT, but not in the isWAT (Fig. 1A and B).

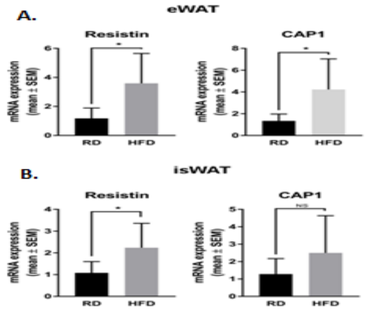


Figure 1. Effect of HFD on resistin and CAP1 gene expression in C57Bl/6J mice. ***eWAT—epididymal WAT, isWAT—intrascapular WAT.*** ** $p < 0.05$.*

HFD affects the expression of cofilin 1 and MMP9 in C57Bl/6J mice

We further investigated the effect of HFD on CAP1 signaling by measuring the expression levels of cofilin 1 mRNA. Results demonstrated that cofilin 1 mRNA levels in isWAT from the mice on HFD were significantly upregulated compared to control (Fig. 2A). We also measured mRNA expression levels of MMP9, which was recently identified as a novel and most efficient substrate of gelatinase B/MMP-9 implying CAP1 degradation *in vivo*[13]. Our results showed that HFD regimen negatively affects MMP9 mRNA expression in isWAT (Fig. 2B).

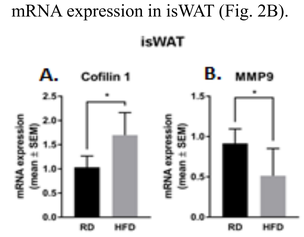


Figure 2. HFD affects the expression of cofilin 1 and MMP9 in C57Bl/6J mice. ***isWAT—intrascapular WAT*** *. * $p < 0.05$.*

Effect of HFD on cell structure proteins expression in C57Bl/6J mice

eWAT and scWAT samples were subjected to Western blot analysis to investigate the expression of resistin, cofilin 1 and α-tubulin proteins. Results demonstrated that HFD group of mice show higher basal expression of resistin and α-tubulin proteins in eWAT and scWAT. In the same time, the protein levels of cofilin 1 were not different between both groups (Fig. 3A

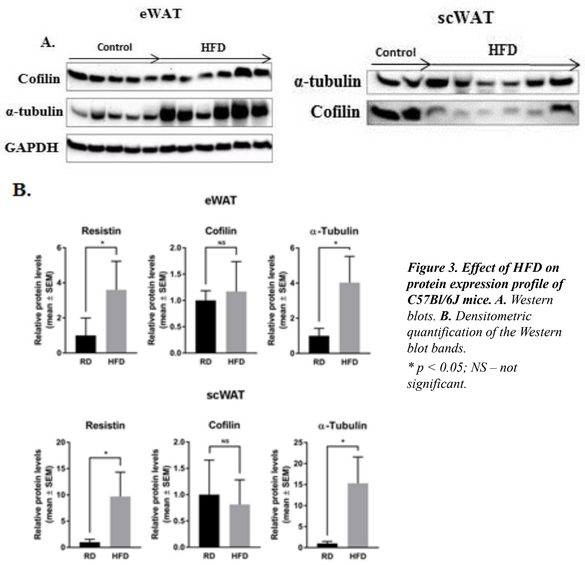


Figure 3. Effect of HFD on protein expression profile of C57Bl/6J mice. A. *Western blots.* ***B.*** *Densitometric quantification of the Western blot bands.*
** $p < 0.05$; NS – not significant.*

Circulating levels of resistin and CAP1 in RD and HDF C57Bl/6J mice

We measured the plasma levels of resistin by using MILLIPLEX analysis and the levels of CAP1 by using ELISA. Results shown that the mice from the HFD group had significantly higher circulating levels of resistin compared to the control RD animals (Fig. 4A). CAP1 levels were not significantly different between the two groups (Fig. 4B).

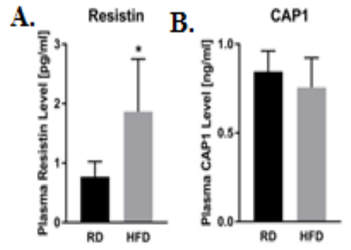


Figure 4. Comparison of resistin and CAP1 plasma levels in RD and HFD C57Bl/6J mice. * $p < 0.05$.

Correlation between plasma levels of resistin and CAP1

Plasma resistin and CAP1 levels were compared individually for each animal. As in the RD group of mice plasma resistin and CAP1 levels did not correlate (Fig. 5A), in the HFD group there was significant negative correlation between resistin and CAP1 (Fig. 5B and C).

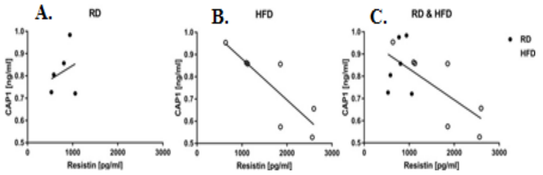


Figure 5. Correlation between plasma levels of resistin and CAP1 in lean and DIO C57Bl/6J mice.

CONCLUSIONS

Data from this study indicate that 9-weeks on 60% HFD regimen in mice leads to an increased WAT expression and secretion into circulation of the proinflammatory cytokine resistin. The levels of CAP1 mRNA were upregulated in eWAT (the adipose tissue mostly affected by inflammation), but plasma levels of CAP1 were not significantly different between the groups. HFD positively affected WAT mRNA expression of cofilin 1, but negatively that of MMP9.
Taken together, these results suggest that resistin could act through CAP1 to induce downstream signaling involving cofilin 1 activation and G-actin fibers reorganization. This could lead to suppression of the GLUT4 glucose transporter translocation from cytoplasm to the extracellular membrane and thus inducing insulin resistance. Simultaneously, HFD suppresses MMP9 production which could be a mechanism for preventing of the cellular CAP1 degradation. Further studies are underway.

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