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Research Article

Central Downregulation of S-Resistin Alleviates Inflammation in EWAT and Liver and Prevents Adipocyte Hypertrophy

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Abstract

The hypothalamus integrates peripheral signals and modulates food intake and energy expenditure by regulating the metabolic function of peripheral tissues, including the liver and adipose tissue. In a previous study, we demonstrated that s-resistin, an intracellular resistin isoform highly expressed in the hypothalamus and upregulated during aging, is important in the central control of energy homeostasis, affecting mainly the peripheral response to insulin by still unknown mechanisms. Herein, using an intracerebroventricular injection of a specific lentiviral RNAi against s-resistin, we assessed, in the Wistar rat, the effects of central s-resistin downregulation on the expression and phosphorylation levels of intermediates involved in insulin signaling and the inflammatory response in epididymal white adipose tissue (eWAT) and liver. Additionally, we studied the imbalance of eWAT hypertrophy/hyperplasia remodeling. Our results indicate that central downregulation of s-resistin regulates insulin signaling cascade in a tissue-specific manner, reduces the inflammatory status both in the liver and eWAT, and prevents eWAT hypertrophy. Taken together, our results highlight the pivotal role of central s-resistin in maintaining metabolic homeostasis in AT and the liver. This suggests a direct association between its function and the modulation of the inflammatory response in these tissues.

Key Words: central-s-resistin, resistin, insulin-resistance, inflammation, hypertrophy

Abbreviations: AKT, protein kinase B; AP-1, activator protein 1; CAP1, cyclase-associated protein 1; cDNA, complementary DNA; CNS, central nervous system; ELISA, enzyme-linked immunosorbent assay; EV, empty virus; eWAT, epididymal white adipose tissue; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; ICV, intracerebroventricular; IL, interleukin; IRβ, insulin receptor β subunit; IRS1, insulin receptor substrate 1; LV, lentivirus; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; NEFA, nonesterified fatty acid; KBs, ketonic bodies; qPCR, quantitative polymerase chain reaction; RNAi-s-res, s-resistin RNAi; TAGs, triglycerides; TLR, toll-like receptor; TNFα, tumor necrosis factor α; WAT, white adipose tissue.

Body weight and global energy homeostasis is controlled by a complex network composed by neuronal circuits of the central nervous system (CNS), peripheral tissues, and hormones such as leptin, insulin, and resistin [1]. The brain modulates food intake and energy expenditure, as well as the metabolic function of peripheral tissues, through the detection of nutritional status. The hypothalamus is the main site for such functions, acting as a signal-integrating center from other brain regions, as well as from peripheral tissues such as adipose tissue and the liver [2].

The endocrine signaling from the white adipose tissue (WAT) to the hypothalamus particularly plays an important role. The deregulation of this adipose-hypothalamic axis produces molecular alterations in the transduction pathways of all these signals, leading to the development of obesity, dyslipidemias, insulin and leptin resistance, and hepatic steatosis [3].

On the other hand, several studies show that hepatic function is controlled by the hypothalamus through the autonomic nervous system as well as by adipokines, hepatokines, and metabolites released by the liver itself. These signals in turn reach the CNS through the blood-brain barrier and modulate the brain response [4, 5].

Under physiological conditions, WAT and liver feedback communication with the hypothalamus maintains the sensitivity of peripheral cells and CNS neurons to hormones and metabolites in a relatively flexible process, buffering small metabolic changes that contribute to the preservation of organism homeostasis [6]. In this sense, WAT can adapt itself in response to different nutritional variations and hypothalamic neuroendocrine signals. In this process, known as the "remodeling of adipose tissue," several qualitative and quantitative changes occur [7]. These

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modifications are associated with a variation in the size and number of adipocytes (hypertrophy/hyperplasia), vascularization, the release of adipokines, and recruitment of immune cells [8]. In fact, resident immune cells in WAT play an important role in the control of the inflammatory state of the tissue, influencing the remodeling processes. Macrophages are the dominant immune cells of WAT, both in number and functionally, involving proinflammatory and anti-inflammatory responses. While AT macrophages contribute to maintaining metabolic homeostasis inside the tissue, a switch from an anti-inflammatory to a proinflammatory macrophage phenotype has been observed in obesity-driven insulin resistance and metabolic disease [8-10]. This balance between proinflammatory and anti-inflammatory states is very sensitive to the energetic conditions of the organism. Indeed, free fatty acids can lead to toll-like receptor (TLR) stimulation in macrophages activating proinflammatory cytokines production such as interleukin 1β (IL-1β), tumor necrosis factor α (TNFα), or interleukin 6 (IL-6). Thus, the proinflammatory cytokines accumulation triggers insulin resistance while reducing plasticity toward an anti-inflammatory macrophage phenotype [11].

One of the hormones related to the regulation of energy metabolism is resistin [12]. This adipokine promotes inflammation, insulin resistance, and cardiovascular diseases as shown in studies with rodents and humans [13]. In addition to its direct actions on peripheral organs, the presence of resistin in the cerebrospinal fluid, as well as in certain hypothalamic nuclei, suggests a role for this adipokine in the adipo-hypothalamic axis [14].

In rodents, resistin acts through the CNS reducing food intake, thermogenesis, and peripheral insulin sensitivity [15, 16]. In addition, central resistin modulates glucose homeostasis and lipid metabolism and generates hepatic insulin resistance [17, 18].

An intracellular resistin isoform, s-resistin, was first described and characterized by our group in WAT from the Wistar rat [19]. This resistin variant is generated by alternative splicing that eliminates the exon 2 that contains the secretion signal. Thus, s-resistin, unlike resistin, is a nonsecreted intracellular resistin isoform. Transfection in vitro experiments in the 3T3-L1 preadipocyte cell line demonstrate that s-resistin has a preferentially nuclear location [19]. Besides this, like resistin, s-resistin inhibits the adipocyte differentiation process but only s-resistin increases the expression and secretion levels of TNFα since the early stages of this process, implicating this resistin variant in the inflammatory stages [20].

Interestingly, s-resistin is very abundant in the hypothalamus, where its expression increases with aging in parallel to the development of inflammation and hypothalamic resistance to insulin and leptin. In addition, we demonstrated that central s-resistin downregulation using an intracerebroventricular (ICV) injection of a specific lentiviral in a physiologically normal model, such as 3-month-old Wistar rats, to gain insight into the physiological function of s-resistin. RNAi reduces hypothalamic and peripheral inflammation and increases whole-body insulin sensitivity. Based on these data, we hypothesized that central s-resistin could have an important role in the control of energy homeostasis, affecting mainly the peripheral response to insulin by still unknown mechanisms [21].

Herein we investigated the peripheral effects of the downregulation of central s-resistin on insulin signaling and inflammation in eWAT and liver, 2 key tissues in the maintenance of energy homeostasis. Moreover, we studied the imbalance of eWAT hypertrophy/hyperplasia remodeling.

Our results indicate that central s-resistin downregulation reduces the inflammatory status in eWAT and the liver and regulates in a tissue-specific manner the insulin sensitivity in both tissues. Additionally, we observed that the downregulation of central s-resistin expression prevents eWAT hypertrophy. Taken together, these data support the hypothesis that central s-resistin isoform could play an important role in the molecular alterations underlying systemic insulin resistance.

Materials and Methods

Animals and Ethical Statement

Male 3-month-old Wistar rats were individually housed and maintained on a 12-hour light/dark cycle at a controlled temperature (20-25 °C) and humidity (50%) with free access to food and water. Animals were fed a standard chow diet (2014 Tekland Global 14% Protein Rodent Maintenance Diet). All animal experiments were conducted according to the European Union laws (2010/63/EU) and following the Spanish regulations (RD 53/2013) for the use of laboratory animals. The experimental protocols were approved by the institutional and regional ethical committees (permit Nos. CE/301012 and CE/99-1835-A308, respectively). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Lentivirus Injection

The animals were treated as previously described [21]. Briefly, 3month-old rats were anesthetized by inhalation of a mixture of O_2 and isoflurane and placed on the stereotaxic apparatus (David Koppf). Rats were injected with lentivirus (LV) containing the antisense sequence selective for s-resistin (RNAi-s-res) or control empty virus (EV) into the left lateral ventricle using the following coordinates, according to Paxinos' atlas: AP: −0,8 mm; L: 1,6 mm; DV: −3,4 mm (RNAi-s-res n = 5; EV n = 5). The viral stock (10^8 TU/mL) was injected at a speed of 1 µL/min with a 10 µL Hamilton syringe. The needle was left in place for an additional 5 minutes to avoid reflux along the injection track. The total volume injected was 5 µL. Rats were returned to the housing colony and humanely killed by decapitation 10 days after LV administration. eWAT fat pad, and the liver were dissected, weighed, and flash-frozen in liquid nitrogen and stored at −70 °C until use. Also, blood samples were centrifuged, and plasma was frozen until use.

Plasma Metabolite and Hormone Analysis

Lactate and glucose were measured in blood using an Accutrend analyzer (Roche). Plasma triglyceride (TAG) levels were determined by an enzymatic kit from Biosystem. Plasma nonesterified fatty acids (NEFAs) and ketone bodies (KBs) were measured with enzymatic kits from WAKO Chemical. Plasma leptin and insulin levels were assayed using specific rat enzyme-linked immunosorbent assay (ELISA) kits from Bertin Farma (catalog No. A05176, RRID: AB_3662873 and catalog No. A05105, RRID: AB_3662874). Plasma resistin was assessed using a rat resistin ELISA kit (BioVendor: catalog No. RD391016200R, RRID: AB_3662921), following the manufacturer's instructions. Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was calculated as fasting insulin (µU/mL) × fasting glucose ([mmol/L]/22.5) [22].

RNA and Protein Isolation

Total RNA from the liver was isolated using RNeasy Mini Kit (Qiagen) and total RNA from adipose tissue was obtained using RNeasy Lipid Tissue Mini Kit (Qiagen) following the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1 µg of DNase-treated RNA [23].

For protein isolation, 100 mg of tissue (liver or eWAT) was homogenized in 1 mL of RIPA buffer (50 mM Tris-HCl, pH 8.0, with 150-mM sodium chloride, 1.0% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) with 2-mM Na_3VO_4, 10 µg/mL leupeptin, 10 µg/mL aprotinin, 10 µg/mL, and pepstatin using a manual Dounce homogenizer. The homogenate was centrifuged at 4500*g* for 15 minutes, and the supernatant was considered as total extract. Protein concentrations were determined using the Bradford protein assay kit (Bio-Rad).

Real-Time Quantitative Polymerase Chain Reaction Analysis

Real-time quantitative polymerase chain reaction (qPCR) was performed by using an ABI PRISM 7500 Fast Sequence Detection System instrument and software (Applied Biosystem). Relative quantification of target cDNA in each sample was performed from 4 ng of cDNA in TaqMan One-Step Real-Time PCR Master Mix and using Pre-Developed FAM TaqMan Assay Reagents (PE Applied Biosystem) for insulin receptor β subunit (IRβ), insulin receptor substrate 1 (IRS1), mammalian target of rapamycin (mTOR), protein kinase B (AKT), IL-6, IL-10, IL-1β, Arg1, CD68, CD32, CD3g, CD64, CCL5, and 18S ribosomal RNA with VIC as real-time reporter, was used as control to normalize gene expression (Supplementary Table S1 [24]). Furthermore, relative quantification of target cDNA in each sample was performed from 4 ng of cDNA in SYBR-Green One-Step real time PCR Master Mix, with primers supplied by Bonsai Technologies, for s-resistin, (sense primer, a s-resistin-specific forward primer derived from exon 1/exon 3 junction [19], resistin, TNFα, TLR4, and ribosomal RNA (18S) (Supplementary Table S2 [24]).

The ΔΔCT method was used to calculate the relative differences between experimental conditions and control groups as a fold change in gene expression [25].

Western Blot Analysis

Proteins (equal amount of 20-30 µg) were fractionated on a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel and blotted. Western Blot (WB) analysis was performed by antibody incubation in phosphate-buffered saline containing 0.05% Tween and 5% (w/v) milk powder according to standard protocols.

The following primary antibodies were used in this study: β-actin (Sigma-Aldrich catalog No. A2228, RRID: AB_476697, 1:1000 dilution), pS-473-AKT (Cell Signaling catalog No. 9271, RRID:AB_329826, 1:200), AKT (Cell Signaling catalog No. 9272, RRID:AB_39827, 1:1000), pY-1146-IRβ (Cell Signaling catalog No. 3021, RRID: AB_331578, 1:500), IRβ (Santa Cruz catalog No. 57342, RRID:AB_784102, 1:500), pY-632-IRS1 (Abcam catalog No. 109543, RRID:AB_10864315, 1:500), IRS1 (Cell Signaling catalog No. 2382, RRID:AB_330333, 1:500), pS-2448-mTOR (Abcam catalog No. 51044, RRID: AB_2247119, 1:1000), mTOR (Abcam catalog No. 2833, RRID:AB_303343, 1:1000), TLR4 (Santa Cruz catalog No. 293072, RRID:AB_10611320, 1:1000), c-Fos (Santa Cruz catalog No. 166940, RRID:AB_2943286, 1:1000), pT-91-93-c-Jun (Santa Cruz catalog No. 822, RRID:AB_627262, 1:500), c-Jun (Santa Cruz catalog No. 166540, RRID: AB_2280720, 1:1000), Goat anti-rabbit IgG-HRP (Sigma catalog No. A0545, RRID:AB_257896), and rabbit anti-Mouse IgG-HRP (Sigma catalog No. A9044, RRID: AB_258431) were used as secondary antibodies, and ECL Prime (Amersham) reagent was used for developing. Bands were quantified by scanning densitometry with a G-Box densitometer with exposure in the linear range using Gene Tools software (Synergy). The relative levels of phosphorylated and total proteins were normalized to the corresponding amount of total protein and β-actin, respectively, in the same sample (See Supplementary Figs. S1A, S1B, S2, and S4 of supplementary data for uncropped WBs [24]).

Determination of Triglyceride Concentration

TAGs were extracted in 1 mL of isopropanol from 40 mg of tissue, using a Potter-Elvehjem–type homogenizer. After 30 minutes of incubation at room temperature, a 2000*g* centrifugation was performed for 5 minutes at 4 °C. Once the TAGs were extracted, their concentration was determined using the Triglycerides kit (Biosystems) following the protocol recommended by the manufacturer. Absorbance measurements at 500 nm were performed with a Synergy HT spectrophotometer (Agilent BioTek, Thermo Fisher Scientific).

Hematoxylin-Eosin Staining: Measurement of Adipocyte Size and Number

Adipocyte size was determined on cryostat 30-µm sections of eWAT stained with hematoxylin and eosin, as previously reported [26]. After staining, to determine the average size of the adipocyte, fat cell Feret diameters were determined in 4 to 6 sections of eWAT from 3-month-old rats treated ICV with RNAi-s-res or EV (n = 100 adipocytes), using the National Institutes of Health Image J image software. The number of adipocytes was counted using AdipoCount software [27]. A Nikon Eclipse 90i optical microscope equipped with a 10× objective and a Nikon DXM1200C camera were used to observe the stained sections and obtain the images to be analyzed.

Statistical Analysis

Statistical significance between RNAi-s-res–treated and control animals was calculated using the *t* test (Graph Pad Prism 9.5, Graph Pad Software Inc). *P* values less than .05 were considered statistically significant.

Results

Downregulation of Central S-Resistin Expression Differentially Regulates Insulin Sensitivity in Epididymal White Adipose Tissue and Liver in the 3-Month-Old Wistar Rat

As shown in Table 1, body weight and fasting plasma glucose levels were not changed in rats with reduced hypothalamic s-resistin expression as previously shown [21]. In these animals, fasting plasma insulin levels were much lower than in control rats, leading to a decrease in HOMA-IR values. Moreover, plasma levels of leptin, resistin and NEFAs were also lower in ICV-RNAi-s-resistin animals. These data suggest an improvement in overall insulin sensitivity and glucose homeostasis

Table 1. General characteristics of the animals

	EV	RNAi-s-res
Body weight, g	374 ± 12	393 ± 13
NEFA, mmol/L	1.24 ± 0.02	1.13 ± 0.03[a]
KBs, mmol/L	0.18 ± 0.03	0.16 ± 0.03
TAGs, mg/dL	118 ± 9	106 ± 9
Glucose, mg/dL	106 ± 8	117 ± 5
Lactate, mmol/L	3.8 ± 0.1	4.2 ± 0.1
Insulin, ng/mL	1.8 ± 0.5	0.84 ± 0.1[a]
Resistin, ng/mL	15 ± 1	10 ± 2[a]
Leptin, ng/mL	4.8 ± 0.3	3.7 ± 0.6[a]
HOMA-IR	12 ± 2	6.1 ± 0.5[a]

Animals injected with the EV and with the virus with RNAi-s-res. Values are means ± SEM; n = 4-5 independent experiments per group. Biochemical determinations were made in duplicate.
Abbreviations: EV, empty virus; NEFA, nonesterified fatty acid; KBs, ketonic bodies; TAGs, triglycerides; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; RNAi-s-res, s-resistin RNAi.
[a]*P* less than or equal to .05 compared to EV (*t* test).

following central s-resistin downregulation. Thus, to determine the effect of central s-resistin downregulation on the insulin signaling pathway in the eWAT and liver of 3-months-old Wistar rats, we analyzed the messenger RNA (mRNA) and protein levels as well as the activation status of several intermediaries of the insulin signaling pathway including IRβ, IRS1, AKT, and mTOR through phosphorylation analysis. Our results show that in eWAT, although there was an increase in IRβ and IRS1 mRNA levels in rats centrally treated with s-resistin-RNAi (Fig. 1A), no changes were detected in the protein content or basal phosphorylation levels of the insulin signaling intermediates pY-1146-IRβ, pS-473-Akt, and pS-2448-mTOR (Fig. 1B).

Nonetheless, a different response was observed in the liver regarding insulin signaling intermediates. In fact, downregulation of central s-resistin increased mRNA levels of IRS1, AKT, and mTOR (see Fig. 1A) as well as basal Y-1146 phosphorylation of IRβ, Y-632 phosphorylation of IRS1, S-473 phosphorylation of AKT, and S-2448 phosphorylation of mTOR (Fig. 1C). These data support an improvement in the insulin signaling pathway in the liver associated with the downregulation of central s-resistin expression.

Downregulation of Central S-Resistin Expression Modify Activator Protein 1 Protein Complex in Liver but not in Epididymal White Adipose Tissue

The activator protein 1 (AP-1) transcription factor members, c-Jun and c-Fos, are major regulators of cell proliferation and differentiation, and it has been described that insulin modulates AP-1 activity through the phosphorylation of these proteins [28]. Therefore, to test the insulin sensitivity in the eWAT and liver of rats infused with RNAi-s-res, we analyzed protein levels and phosphorylation status of c-Jun and c-Fos. As shown in Fig. 2A, the downregulation of central s-resistin significantly increased c-Fos protein and basal T-91-93-c-Jun phosphorylation levels in the liver. However, no changes were observed in the c-Fos protein or basal T-91-93 of c-Jun phosphorylation levels in eWAT (Fig. 2B). These results confirm that the decrease in central s-resistin levels improves insulin sensitivity only in the liver, with no effect in the eWAT, suggesting a different role of s-resistin in these two tissues.

Downregulation of Central S-Resistin Levels Reduces the Inflammatory State in Epididymal White Adipose Tissue and Liver From 3-Month-Old Wistar Rats and Reduces the Immune Cell Infiltration in Epididymal White Adipose Tissue

Inflammation is directly associated with insulin sensitivity. Previously, we have reported that the ICV infusions of RNAi-s-res reduce peripheral inflammation by decreasing plasma levels of proinflammatory cytokines [21]. To go deeper into the effects of diminished expression levels of hypothalamic s-resistin on the peripheral tissue inflammatory response, we determine the expression levels of diverse inflammation-related signaling genes such as IL-1β, IL-6, TNF-α, and IL-10 in the liver and eWAT of treated animals. Furthermore, as resistin and s-resistin are also linked with inflammation and insulin resistance states [20, 29, 30], the mRNA levels were also measured. As shown in Fig. 3A and 3B, downregulation of central s-resistin levels led to a reduction in the expression of IL-1β, TNF-α, and IL-10 in both tissues, and IL-6 only in the liver, as well as mRNA levels of s-resistin in the liver and the two resistin variants in eWAT (Fig. 3C and 3D). These results confirm the anti-inflammatory effects on peripheral tissues resulting from the central reduction of s-resistin levels.

Additionally, TLR4 has been proposed to be responsible for the molecular mechanisms involved in resistin-induced inflammation and insulin resistance [5]. However, central s-resistin downregulation does not significantly change TLR4 mRNA or protein levels in both tissues (Supplementary Fig. S3 [24]).

Furthermore, to explore whether the reduction in the observed inflammatory state in eWAT and liver is associated with changes in the recruitment of immune cells in these tissues, we analyzed the expression of specific markers of immune cell subpopulations as macrophages (CD68), anti-inflammatory macrophages (Arg1), lymphocytes B (CD32), and lymphocytes T (CD3g) in the eWAT and liver of rats with decreased central s-resistin levels. As shown in Fig. 3E and 3F, we observe a reduction in the expression of the Arg1/CD68 ratio in both tissues, while only in eWAT, we observed a decrease in B lymphocytes (see Fig. 3F). The same expression pattern was also observed in other immune cell markers analyzed as CD64 and CCL5 in both tissues (Supplementary Fig. S5 [24]). These findings suggest that the central reduction in s-resistin leads to decreased immune cell infiltration in eWAT, which in turn reduces inflammatory signals in this tissue.

Downregulation of Central S-Resistin Prevents Adipocyte Hypertrophy and Promotes Hyperplasia

Considering the reduced recruitment of immune cells observed in eWAT, we aimed to analyze the effect of the central decrease of s-resistin on the balance between hypertrophy and hyperplasia. The results indicate that although there are no changes in the TAG content between animals centrally treated with ICV-RNAi-s-res and control animals (Fig. 4A), we observed a statistically significant reduction in the size of the eWAT adipocytes in the animals treated with RNAi-s-res (Fig. 4B) concurrent with an increment in the number of adipocytes (Fig. 4C) These findings suggest that the decrease in central s-resistin prevents eWAT hypertrophy and promote hyperplasia.

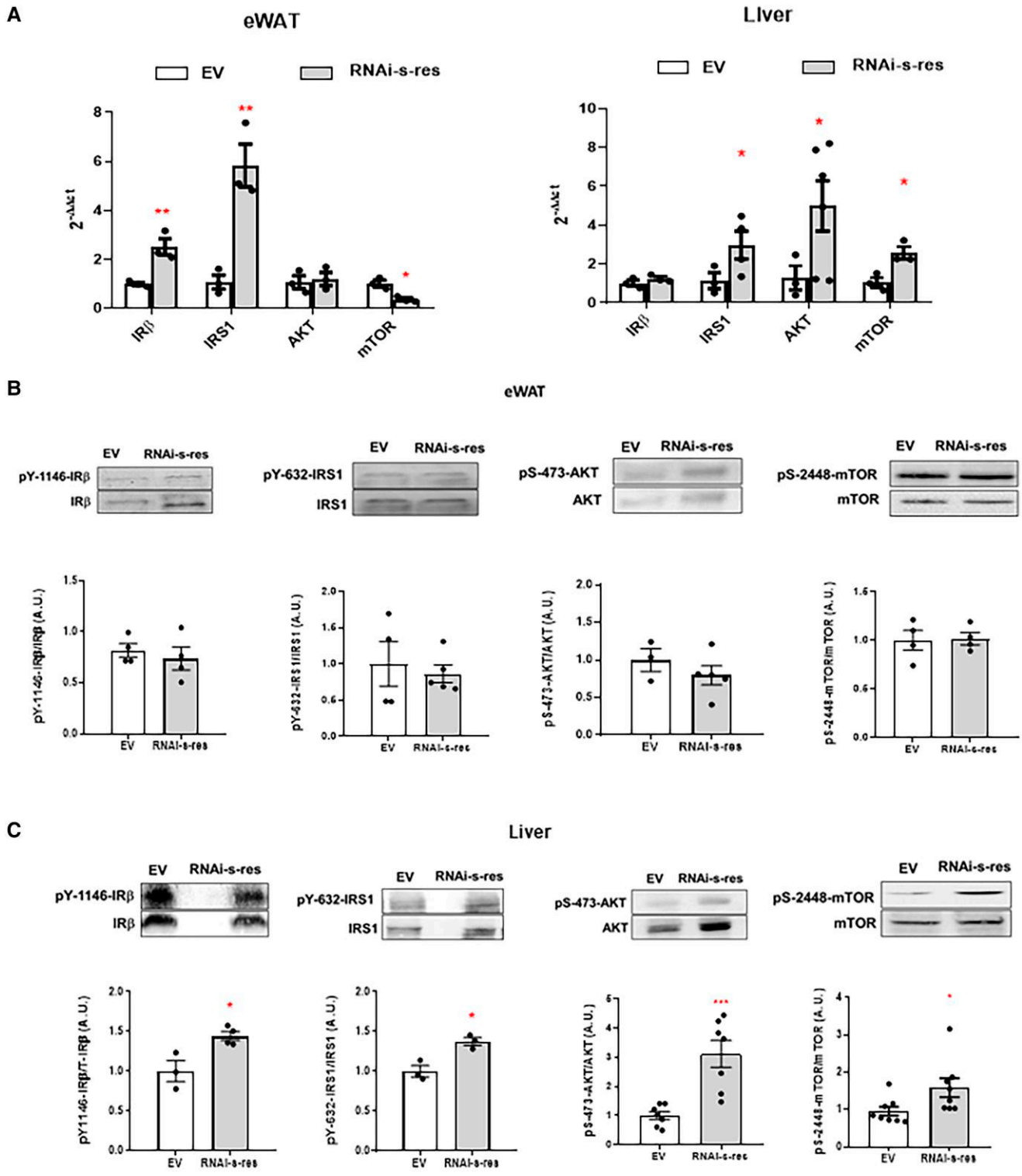

Figure 1. LV-RNAi-s-res central administration differentially regulates insulin sensitivity in epididymal white adipose tissue (eWAT) and liver in a 3-month-old Wistar rat. A, Expression levels of IRβ, IRS1, protein kinase B (AKT), and mammalian target of rapamycin (mTOR) in the eWAT and liver of intracerebroventricular (ICV)-treated animals analyzed by quantitative polymerase chain reaction. B, Representative WB of protein levels and Y-1146 phosphorylation of IRβ, Y-632 phosphorylation of IRS1, S-473 phosphorylation of AKT, and S-2448 phosphorylation of mTOR, as well as phosphorylated protein vs total protein ratios determined by optical density analysis of the eWAT of ICV-treated animals. C, Representative Western blot (WB) of protein levels and Y-1146 phosphorylation of IRβ, Y-632 phosphorylation of IRS1, S-473 phosphorylation of AKT, and S-2448 phosphorylation of mTOR, as well as phosphorylated protein vs total protein ratios determined by optical density analysis of the liver of ICV-treated animals. Values are the mean ± SEM; n = 4-5 separated determinations per group of animals, each sample made in duplicate. **P* less than .05 compared to empty virus (EV) (*t* test). Animal injected with the EV; animal injected with virus with s-resistin RNAi (RNAi-s-res).

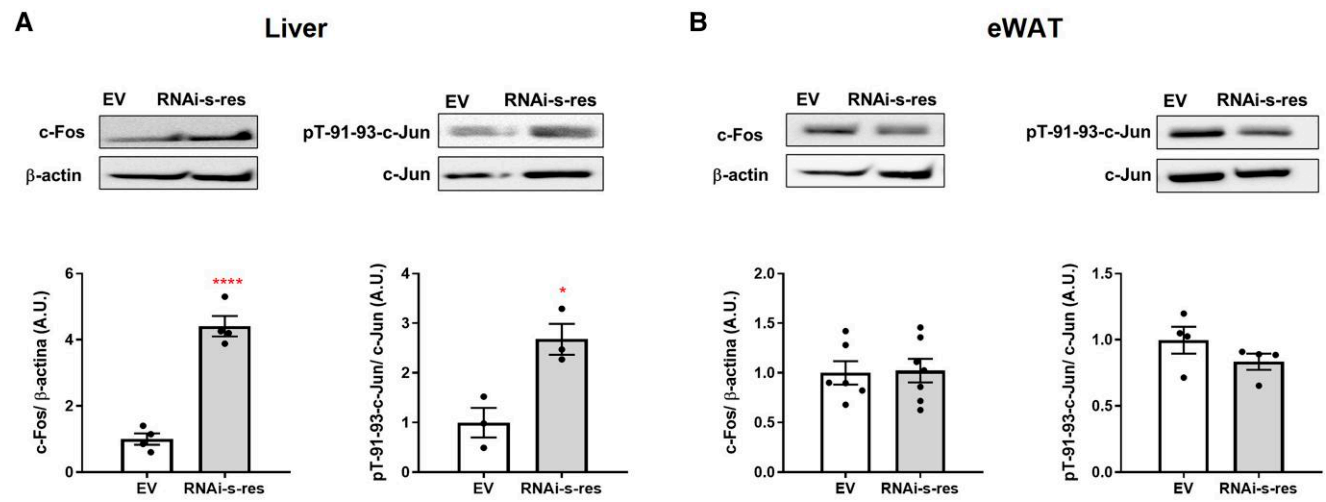

Figure 2. Effect of downregulation of central s-resistin expression on AP-1 pathway in epididymal white adipose tissue (eWAT) and liver in a 3-month-old rat. A, Representative Western blot (WB) of protein levels of c-Fos and protein and pT-91-93 phosphorylation of c-Jun, as well as c-Fos/β-actin and pT-91-93-c-Jun/c-Jun ratios determined by optical density analysis of the liver of intracerebroventricular (ICV)-treated animals. B, Representative WB of protein levels of c-Fos and protein and pT-91-93 phosphorylation of c-Jun, as well as c-Fos/β-actin and pT-91-93-c-Jun/c-Jun ratios determined by optical density analysis of the eWAT of ICV-treated animals. Values are mean ± SEM; n = 4-5 separated determinations per group of animals, each sample made in duplicate. *P less than .05 compared to empty virus (EV) (t test). Animal injected with EV, animal injected with virus with s-resistin RNAi (RNAi-s-res).

Discussion

It has been demonstrated that s-resistin, an intracellular resistin-spliced variant [19], is highly expressed in the eWAT of the Wistar rat. This isoform increases insulin resistance, stimulates the secretion of proinflammatory cytokines like TNFα and IL-6, and reduces glucose transport in adipocytes 3T3-L1 [20, 30]. Furthermore, s-resistin is highly expressed in the hypothalamus and its RNA levels increase with aging [21]. This suggests that this isoform could be important in the development of insulin resistance associated with aging since a reduction of hypothalamic inflammation related to an increase of central and peripheral insulin sensitivity is observed when central s-resistin levels are decreased by the ICV infusion of a specific RNAi LV (LV-RNAi-s-res) in 3-month-old adult rats but not in 24-month-old aged rats [21]. It is possible that the LV infusion did not effectively reduce the elevated high central s-resistin levels in aged animals. Additionally, considering that the increase in s-resistin during aging may be linked to leptin and insulin resistance, our main focus is to understand the effects of central s-resistin in a physiologically normal model.

This work aimed to study the specific response of peripheral tissues, specifically the eWAT and liver, in adult Wistar rats with decreased central s-resistin levels, to find out their contribution to the peripheral increase in insulin sensitivity and its relationship with the improvement in the inflammatory response previously [21].

Herein downregulation of central s-resistin reduces inflammatory response both in the eWAT and liver. A statistically significant decrease both in proinflammatory (IL-1β and TNFα) and anti-inflammatory cytokines (IL-10), together with a decrease in the expression of immune cell markers, were observed in the eWAT of the animals with diminished central s-resistin expression. Moreover, our studies showed that the central LV-RNAi-s-res treatment reduces the eWAT adipocyte size and increases the number of adipocytes, without variation in the TAG content. Since adipocyte hypertrophy is related to an increase both in proinflammatory cytokines and immune cell recruitment [7], it seems that the central downregulation of s-resistin levels decreases the inflammatory state in eWAT and could prevent adipose tissue hypertrophy promoting hyperplasia. Likewise, this result is consistent with the fact that the eWAT weight/body weight ratio was decreased in treated animals [21]. The regulation of hyperplasic adipocytes exerts beneficial effects against adipocyte hypertrophy and subsequent insulin resistance [7]. Hyperplasia of adipose tissue is generally considered healthy and adaptive. Several studies have suggested that small adipocytes are especially important for counteracting obesity-associated metabolic decline and the susceptibility to developing diabetes decrease [31].

Besides this, our results also indicate a statistically significant reduction in the mRNA levels of resistin and s-resistin in eWAT, previously described to promote the inflammatory response both in vitro and in vivo [20, 21]. The downregulation of both isoforms (resistin and s-resistin) could also explain the decrease in serum resistin levels of animals treated with LV-RNAi-s-res described previously [21]. In addition, TLR4 has been proposed to be responsible for the molecular mechanisms involved in resistin-induced inflammation and insulin resistance [15]. Although no changes were observed in the expression and protein levels of TLR4 in eWAT, the decrease in serum resistin and NEFA levels in the animals treated with LV-RNAi-s-res [21] could explain the significant decrease in the expression of inflammatory cytokines in eWAT obtained in our analysis. NEFA levels were positively correlated with adiposity, insulin resistance, and diabetes. Therefore, in our model, the improvement in leptin signaling could possibly be responsible for the serum NEFA decrease.

Although the same reduction in the expression of mRNA levels of cytokines was observed in the liver of the animals with lower levels of central s-resistin, along with a diminution of the hepatic s-resistin expression, no changes were observed in B- and T-lymphocyte markers in the livers of these animals, indicating a tissue-specific effect of central s-resistin downregulation on the inflammatory response. At this point, it is important to note that s-resistin, as resistin, is a cytokine mostly

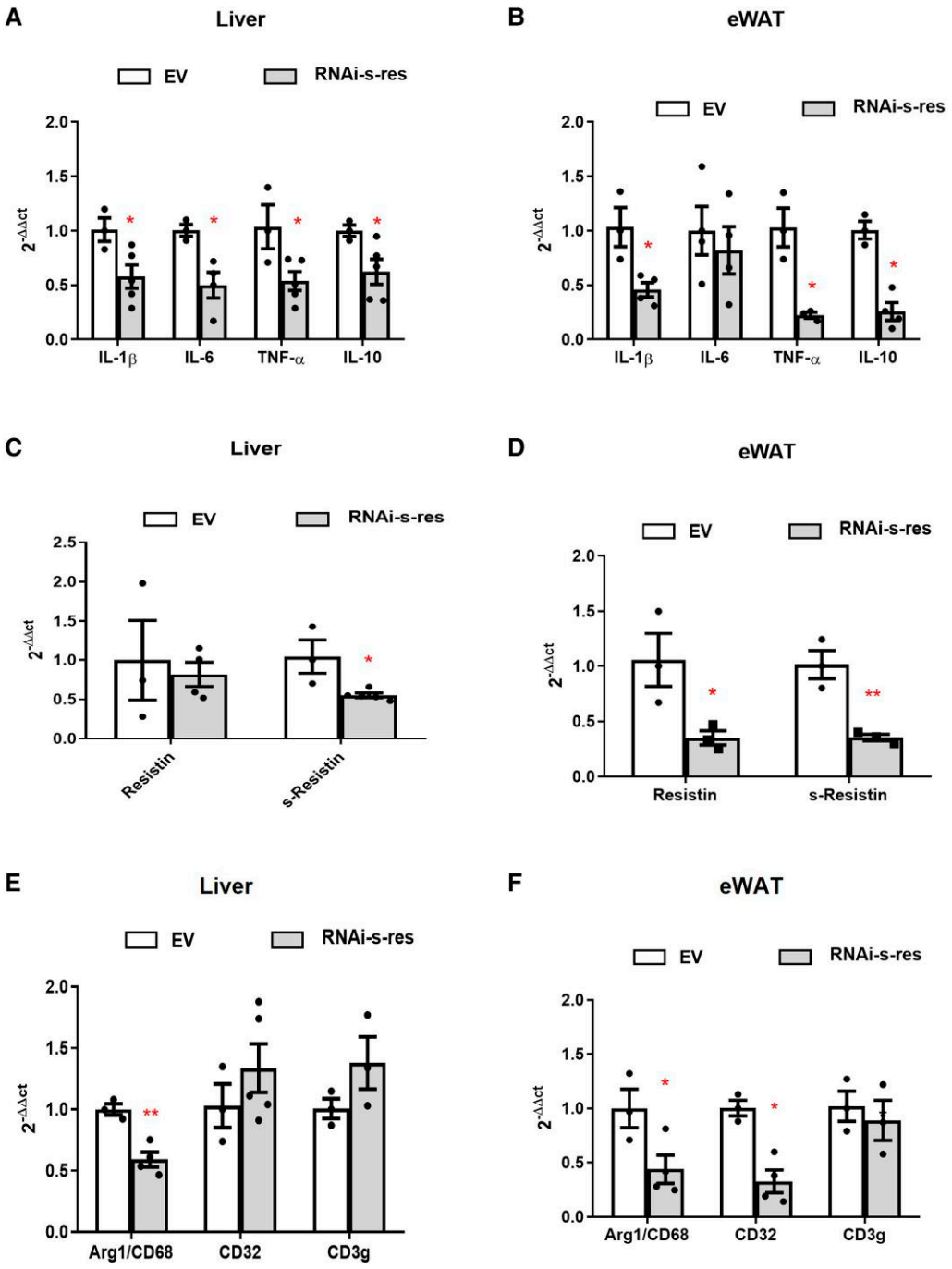

Figure 3. Downregulation of central s-resistin levels reduces the inflammatory state in epididymal white adipose tissue (eWAT) and liver from a 3-month-old Wistar rat, and reduces the immune cell infiltration in eWAT. A, Expression levels of interleukin (IL)-1β, IL-6, tumor necrosis factor α (TNFα), and IL-10 in the liver of intracerebroventricular (ICV)-treated animals analyzed by quantitative polymerase chain reaction (qPCR). B, Expression levels of IL-1β, IL-6, TNFα, and IL-10 in the eWAT of ICV-treated animals analyzed by qPCR. C, Expression levels of resistin and s-resistin in the liver of ICV-treated animals analyzed by qPCR. D, Expression levels of resistin and s-resistin in the eWAT of ICV-treated animals analyzed by qPCR. E, Expression levels of Arg1/CD68 ratio, CD32, and CD3g in the liver of ICV-treated animals analyzed by qPCR. F, Expression levels of Arg1/CD68 ratio, CD32, and CD3g in the eWAT of ICV-treated animals analyzed by qPCR. Values are the mean ± SEM; n = 4-5 separated determinations per group of animals, each sample made in duplicate. * *P* less than .05 compared to empty virus (EV) (*t* test). Animal injected with EV; animal injected with virus with s-resistin RNAi (RNAi-s-res).

expressed in WAT, and its expression in the liver of 3-month-old Wistar rats is very low, almost residual [21]. Although ICV treatment still decreases it further, we do not think that it could have any relevance at the tissue level.

Next, we analyzed the insulin pathway in the eWAT and liver in our model. Our results show that the downregulation of central s-resistin differently modulates, directly or indirectly, the insulin signaling pathway in both tissues.

Thus, in eWAT, although mRNA levels of IRβ and IRS1 are increased with the central reduction of s-resistin, no changes in activation of pathway effectors were observed. It has been widely described that proinflammatory cytokines such as

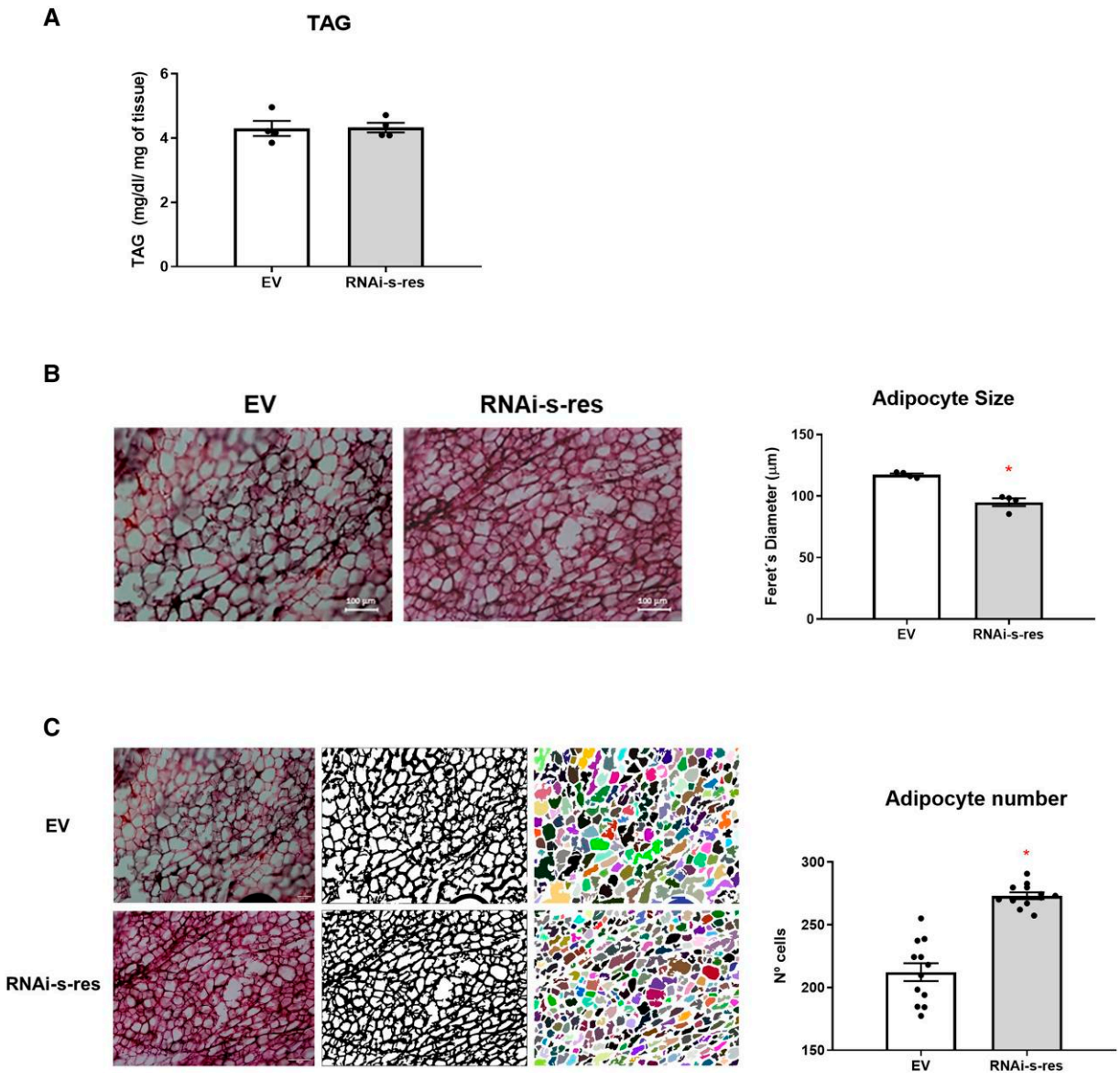

Figure 4. Central s-resistin downregulation prevents adipocyte hypertrophy. A, Plasma triglyceride (TAG) levels in the epididymal white adipose tissue (eWAT) of intracerebroventricular (ICV)-treated animals analyzed by colorimetric assay. B, Hematoxylin-eosin staining of cryostat sections of eWAT and determination of the size of the adipocytes calculating fat cell Feret diameters of 100 adipocytes of ICV-treated animals. C, Hematoxylin-eosin staining of cryostat sections of eWAT and determination of the number of adipocytes using AdipoCount software. Values are the mean ± SEM; n = 4-5 separated determinations per group of animals, each sample made in duplicate. **P* less than .05 compared to empty virus (EV) (*t* test). Animal injected with EV; animal injected with virus with s-resistin RNAi (RNAi-s-res).

TNFα induce the proapoptotic activity of the proliferative transcription factors c-Jun and c-Fos, which form the AP-1 regulatory complex [32]. Despite a decrease in the expression of proinflammatory cytokines and immune cell markers found in eWAT, no alterations in the c-Fos levels and c-Jun phosphorylation (or AP-1 activity) were observed when central s-resistin was downregulated.

By contrast, a different response occurs in the liver. Hence, the liver showed an increase of basal phosphorylation of insulin signaling intermediates after LV-RNAi-s-res treatment, accompanied by an increase in AP-1 activity, which suggests an improvement in insulin sensitivity. Besides apoptosis, AP-1 regulates a wide range of cellular processes, including cell proliferation, differentiation, and cell survival [28, 33], allowing cells to adapt to environmental changes [34]. In addition, it has been described that AP-1 is activated by the insulin pathway [28]. Therefore, our results support a protective role for AP-1 complex in the liver of animals with decreased central s-resistin levels, although we cannot conclusively state an improved tissue response to insulin without analyzing the pathway after insulin injection.

Taken together, our results indicate that the decrease of central s-resistin levels has different effects on eWAT and the liver. In fact, eWAT shows minor basal phosphorylation levels of the insulin signaling intermediates accompanied by a decrease in the inflammatory state and the immune cell recruitment, all this related to small adipocyte size. These effects could lead to tissue remodeling, protecting the adipocyte, and preventing adipose tissue expansion [35]. In a similar context, a comparable effect has been described for leptin. Leptin acting at the

central level enhances insulin sensitivity in peripheral target tissues, except in the case of eWAT, where leptin inhibits the insulin-stimulated glucose uptake, which would contribute to regulate glucose availability for TAG formation and accumulation in this tissue, ultimately contributing to the control of adiposity [36, 37].

Several receptors have been described as putative targets of resistin. In this line, resistin exerts its central functions through sympathetic nervous system activity [36, 38]. Thus, it has been proposed that resistin wields its central functions through TLR4 and adenylyl cyclase-associated protein 1 (CAP1) receptors [15, 39], which activate different signaling cascades implicated in promoting inflammatory processes and insulin resistance. The mechanism by which s-resistin modulates the inflammatory response is still not clarified, but, since s-resistin is an intracellular resistin isoform, surely the transduction signal (or cellular functions) of s-resistin could be through the activation of the same signaling pathways activated by resistin but acting intracellularly. Among the presumed pathways, CAP1 receptor is likely to be the suitable target for s-resistin. CAP1 is a cytoplasmic protein mainly associated with the plasma membrane [39]. Thus, possibly the intracellular location of s-resistin would trigger the signaling pathways regulated by this receptor. On the other hand, the nuclear localization of this resistin variant suggests that it can probably exert its cellular functions by acting as a possible transcription factor or by modulating the activity of any. However, more studies are necessary to clarify this point.

Conclusion

Our results suggest that hypothalamic s-resistin plays a crucial role in the communication between the hypothalamus, adipose tissue, and liver and that its function could be directly related to the modulation of the inflammatory response in both tissues, adipose tissue and liver. Furthermore, as described for the resistin isoform [18, 40], central s-resistin could be responsible for the development of insulin resistance in the liver, but in the eWAT could have a different function, promoting immune cell recruitment, adipocyte hypertrophy, and adipose tissue expansion. These are features observed in our aging model where central levels of s-resistin are upregulated compared to adult animals [21]. However, it is important to note that insulin sensitivity and the inflammatory process are multifactorial events requiring complex studies. The role of s-resistin cannot be studied apart from other hormones and factors that regulate insulin resistance and the inflammatory response such as leptin.

This study in a 3-month-old rat model allows us to examine the direct effects of reducing central s-resistin on peripheral tissues without being influenced by altered prior physiological conditions, like aging or metabolic syndrome and others. By studying the effect of s-resistin at this stage, we are characterizing the early involvement in the resistance process of this short-resistin isoform. Indeed, incorporating a sex perspective is essential. This work represents a preliminary study evaluating the central role of s-resistin in peripheral metabolism. Further studies are necessary to gain a deeper understanding of s-resistin function in both sexes.

Based on our previous results [21] and the findings presented herein, we are tempted to propose that s-resistin can modulate the inflammatory response in the brain by activating resistin-related intracellular pathways and impairing central sensitivity to insulin and leptin. The resulting peripheral effects are tissue-dependent as previously described for central leptin function [35, 36], contributing to the overall impairment of peripheral insulin sensitivity.

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Disclosures

The authors have nothing to disclose.

Data Availability

Some or all data sets generated during and/or analyzed during this study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Sisley S, Sandoval D. Hypothalamic control of energy and glucose metabolism. *Rev Endocr Metab Disord*. 2011;12(3):219-233.
2. Lam TK, Schwartz GJ, Rossetti L. Hypothalamic sensing of fatty acids. *Nat Neurosci*. 2005;8(5):579-584.
3. Ahima RS. Central actions of adipocyte hormones. *Trends Endocrinol Metab*. 2005;16(7):307-313.
4. Imbernon M, Beiroa D, Vazquez MJ, *et al*. Central melanin-concentrating hormone influences liver and adipose metabolism via specific hypothalamic nuclei and efferent autonomic/JNK1 pathways. *Gastroenterology*. 2013;144(3):636-649.
5. Liang Q, Zhong L, Zhang J, *et al*. Fgf21 maintains glucose homeostasis by mediating the cross talk between liver and brain during prolonged fasting. *Diabetes*. 2014;63(12):4064-4075.
6. Smith RL, Soeters MR, Wüst RCI, Houtkooper RH. Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. *Endocr Rev*. 2017;39(4):489-517.
7. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. *Front Endocrinol*. 2016;7:1-16.
8. Pellegrinelli V, Carobbio S, Vidal-Puig A. Adipose tissue plasticity: how fat depots respond differently to pathophysiological cues. *Diabetologia*. 2016;59(6):1075-1088.
9. Hill DA, Lima HW, Kima YH, *et al*. Distinct macrophage populations direct inflammatory versus physiological changes in adipose tissue. *Proc Natl Acad Sci U S A*. 2018;115(22):5096-5105.
10. Kolliniati O, Ieronymaki E, Vergadi E, Tsatsanis C. Metabolic regulation of macrophage activation. *J Innate Immun*. 2022;14(1):51-67.
11. Chawla A, Nguyen KD, Goh YPS. Macrophage-mediated inflammation in metabolic disease. *Nat Rev Immunol*. 2012;11(11):738-749.

12. Steppan CM, Bailey ST, Bhat S, *et al.* The hormone resistin links obesity to diabetes. *Nature*. 2001a;409(6818):307-312.
13. Ahima RS, Lazar MA. Adipokines and the peripheral and neural control of energy balance. *Mol Endocrinol*. 2008;22(5):1023-1031.
14. Kos K, Harte AL, da Silva NF, *et al.* Adiponectin and resistin in human cerebrospinal fluid and expression of adiponectin receptors in the human hypothalamus. *J Clin Endocrinol Metab*. 2007;92(3):1129-1136.
15. Benomar Y, Gertler A, Lacy D, *et al.* Central resistin overexposure induces insulin resistance through toll-like receptor 4. *Diabetes*. 2013;62(1):102-114.
16. Alsuhaymi N, Habeeballah H, Stebbing MJ, Badoer E. High fat diet decreases neuronal activation in the brain induced by resistin and leptin. *Front Physiol*. 2017;8(867):1-13.
17. Vazquez MJ, Gonzalez CR, Varela L, *et al.* Central resistin regulates hypothalamic and peripheral lipid metabolism in a nutritional-dependent fashion. *Endocrinol*. 2008;149(9):4534-4543.
18. Singhal NS, Lazar MA, Ahima RS. Central resistin induces hepatic insulin resistance via neuropeptide Y. *J Neurosci*. 2007;27(47):12924-12932.
19. Del Arco A, Peralta S, Carrascosa JM, Ros M, Andres A, Arribas C. Alternative splicing generates a novel non-secretable resistin isoform in Wistar rats. *FEBS Lett*. 2003;555(2):243-249.
20. Fernández CM, del Arco A, Gallardo N, *et al.* S-resistin inhibits adipocyte differentiation and increases TNF-alpha expression and secretion in 3T3-L1 cells. *Biochim Biophys Acta*. 2010;1803(10):1131-1141.
21. Rodriguez M, Pintado C, Moltó E, *et al.* Central s-resistin deficiency ameliorates hypothalamic inflammation and increases whole body insulin sensitivity. *Sci Rep*. 2018;8(1):3921.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-419.
23. Peralta S, Carrascosa JM, Gallardo N, Ros M, Arribas C. Ageing increases SOCS-3 expression in rat hypothalamus: effects of food restriction. *Biochem Biophys Res Commun*. 2002;296(2):425-428.
24. Rodríguez M, Moltó E, Serrano R, *et al.* Supplementary Data from Central downregulation of s-resistin alleviates inflammation in eWAT and liver and prevents adipocyte hypertrophy. *Digital Repository RUIdeRA*, Date of deposit 19 October 2024. https://hdl.handle.net/10578/38881
25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta CT). *Method. Methods*. 2001;25(4):402-408.
26. Bonzón-Kulichenko E, Moltó E, Pintado C, *et al.* Changes in visceral adipose tissue plasma membrane lipid composition in old rats are associated with adipocyte hypertrophy with aging. *J Gerontol A Biol Sci Med Sci*. 2018;73(9):1139-1146.
27. Zhi X, Wang J, Lu P, Jia J, Shen H-B, Ning G. Adipocount: a new software for automatic adipocyte counting. *Front Physiol*. 2018;9:1-9.
28. Backes TM, Langfermann DS, Lesch A, *et al.* Regulation and function of AP-1 in insulinoma cells and pancreatic β-cells. *Biochem Pharmacol*. 2021;193(114748):1-12.
29. Tripathi D, Kant S, Pandey S, Ehtesham NZ. Resistin in metabolism, inflammation, and disease. *FEBS J*. 2022;287(15):3141-3149.
30. Rodríguez M, Moltó E, Aguado L, *et al.* S-resistin, a non secretable resistin isoform, impairs the insulin signalling pathway in 3T3-L1 adipocytes. *J Physiol Biochem*. 2015;71(3):381-390.
31. Ghaben AL, Scherer PE. Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol*. 2019;20(4):242-258.
32. Yue J, López JM. Understanding MAPK signaling pathways in apoptosis. *Int J Mol Sci*. 2020;21(7):2346.
33. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol*. 2002;4(5):131-136.
34. Bejjania F, Evanno E, Zibara K, Piechaczyk M, Jariel-Encontre I. The AP-1 transcriptional complex: local switch or remote command? *Biochim Biophys Acta Rev Cancer*. 2019;1872(1):11-23.
35. Bonzón-Kulichenko E, Fernández-Agulló T, Moltó E, *et al.* Regulation of insulin-stimulated glucose uptake in rat white adipose tissue upon chronic central leptin infusion: effects on adiposity. *Endocrinology*. 2011;152(4):1366-1377.
36. Gallardo N, Bonzón-Kulichenko E, Fernández-Agulló T, *et al.* Tissue-Specific effects of central leptin on the expression of genes involved in lipid metabolism in liver and white adipose tissue. *Endocrinology*. 2007;148812(12):5604-5610.
37. Chouchani ET, Kajimura S. Metabolic adaptation and maladaptation in adipose tissue. *Nat Metab*. 2019;1(2):189-200.
38. Badoer E, Kosari S, Stebbing MJ. Resistin, an adipokine with non-generalized actions on sympathetic nerve activity. *Front Physiol*. 2015;6:321.
39. Lee S, Lee H-C, Kwon Y-W, *et al.* Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. *Cell Metab*. 2014;19(3):484-497.
40. Muse ED, Lam TKT, Scherer PE, Rossetti L. Hypothalamic resistin induces hepatic insulin resistance. *J Clin Invest*. 2007;117(6):1670-1678.