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ORIGINAL ARTICLE

SRC-3 deficient mice developed fat redistribution under high-fat diet

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Abstract An emerging concept suggests that an aberrant distributed body fat is closely linked to the occurrence of metabolic abnormalities. Mice deficient in steroid receptor coactivator-3 (SRC-3) are shown to be protected against high-fat diet (HFD) induced obesity but little is known about whether visceral (VAT) and subcutaneous adipose tissue (SCAT) distribute differently in SRC-$3^{-/-}$ mice versus SRC-$3^{+/+}$ mice. Here we reported that under HFD, fat redistributed between VAT and subcutaneous area of SRC-$3^{-/-}$ mice. When VAT/SCAT weight ratio (VAT/SCAT ratio) was calculated, SRC-$3^{-/-}$ mice had significantly elevated VAT/SCAT ratio in HFD versus normal diet (ND), while VAT/SCAT ratio was similar in SRC-$3^{+/+}$ mice under ND and HFD. Serological changes in SRC-$3^{-/-}$ mice paralleled the altered fat distribution. In SRC-$3^{-/-}$ mice, assays on gene expression revealed an increase in adipogenesis in VAT versus SCAT and an elevation in thermogenesis and lipolysis in SCAT versus VAT, which could explain the preferential fat accumulation in SRC-$3^{-/-}$ VAT. Our results presented in vivo evidence that SRC-3 deficiency could lead to fat redistribution under HFD in mice and provided new clues to researches on the pathogenesis of fat redistribution.

Keywords Steroid receptor coactivator-3 · Visceral fat · Subcutaneous fat · High-fat diet · Fat redistribution

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Introduction

Steroid receptor coactivator-3 (SRC-3) is a member of the p160 family of nuclear receptor coactivators [1]. When recruited by transcription factors, SRC-3 is capable of recruiting histone acetyltransferases, thus interacts directly with general transcription machines and acts as a platform for other coactivators to function uniformly to start target gene transcription [2]. Physiologically, SRC-3 has substantial functions in regulating mammary gland development [3], puberty onset, cell proliferation [4], and inflammation [5]. SRC-3 has also been designated as an oncogene for its frequent amplification in various cancer cells [6–9].

Besides its role in multiple biological processes, recent researches have shed light on the impact of SRC-3 in the control of adipogenesis as well as energy homeostasis. Bert O'Malley's group showed that SRC-3 functioned as a critical regulator of white adipocyte development by facilitating the gene expression of peroxisome proliferator-activated receptor $\gamma 2$ (PPAR$\gamma 2$), a master gene required for adipogenesis [10]. As for the metabolic features of SRC-3, it is reported that SRC-$3^{-/-}$ mice are protected against high-fat diet (HFD) induced obesity due to enhanced energy expenditure [11]. Similarly, mice lacking both SRC-1 and SRC-3 are lean and exhibit an increased basal metabolic rate [12].

Although preliminary profiles about fat accumulation in SRC-3$^{-/-}$ mice under normal diet (ND) and HFD have been given, it remains undetermined whether SRC-3 deficiency has different impacts on the accretion of visceral (VAT) and subcutaneous adipose tissue (SCAT). Investigation concerning this aspect would be interesting and necessary because, as revealed by clinical studies, compared to the extent of total body fat accumulation, the abnormal percent change in VAT fat versus subcutaneous fat (also called fat redistribution) is a more important determinant factor in the development of obesity-related diseases and morbidities [13, 14]. Seiichiro Tarui's and Claude Bouchard's group both demonstrated that obese women with a preferential VAT fat accumulation were more tightly related to the occurrence of diabetes mellitus and hyperlipidemia than those with a normal fat distribution [15, 16]. Even in mildly obese subjects, as clarified by Yuji Matsuzawa, increased VAT fat accumulation is significantly correlated with coronary artery disease occurrence [17].

With the increasing recognition of aberrant body fat redistribution as a major metabolic risk factor, intensive research emphasis is focusing on the intrinsic mechanism that causes the unbalanced accumulation between VAT and SCAT. Among several scientific exploring fields, animal models with a fat redistribution property have particular physiological significance because of the in vivo evidence they present. For example, Nicholas Morton demonstrated that 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) knockout mice had reduced VAT fat accumulation upon high-fat feeding while Hiroaki Masuzaki found that transgenic mice overexpressing 11β-HSD-1 selectively in adipose tissue developed VAT obesity that was exaggerated by a HFD. It is thus concluded that levels of 11β-HSD-1 could alter adipose tissue distribution and function [18, 19]. This promoted us to elucidate the role of SRC-3 on fat distribution using SRC-3 knockout mice.

In this study, we reported that although SRC-3$^{-/-}$ mice are resistant to HFD induced obesity, proportionally, they accumulated more fat in VAT area and less in subcutaneous as compared to SRC-3$^{+/+}$ mice. Serological changes in SRC-3$^{-/-}$ mice and their wild-type littermates paralleled the difference in the body fat distribution of two genotypes. The fat redistribution in SRC-3$^{-/-}$ mice under energy challenge could be attributed to the different expression levels of adipogenic, thermogenic, and lipolytic marker genes in VAT and SCAT in the absence of SRC-3.

Results

Fat redistributed in SRC-3$^{-/-}$ mice under energy challenge

Under both ND and HFD, SRC-3$^{-/-}$ mice weighed significantly less than SRC-3$^{+/+}$ mice (Fig. 1a, b). To investigate the impact of SRC-3 deficiency on body fat distribution, VAT and SCAT of SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice were dissected, weighed, and normalized by total body weight.

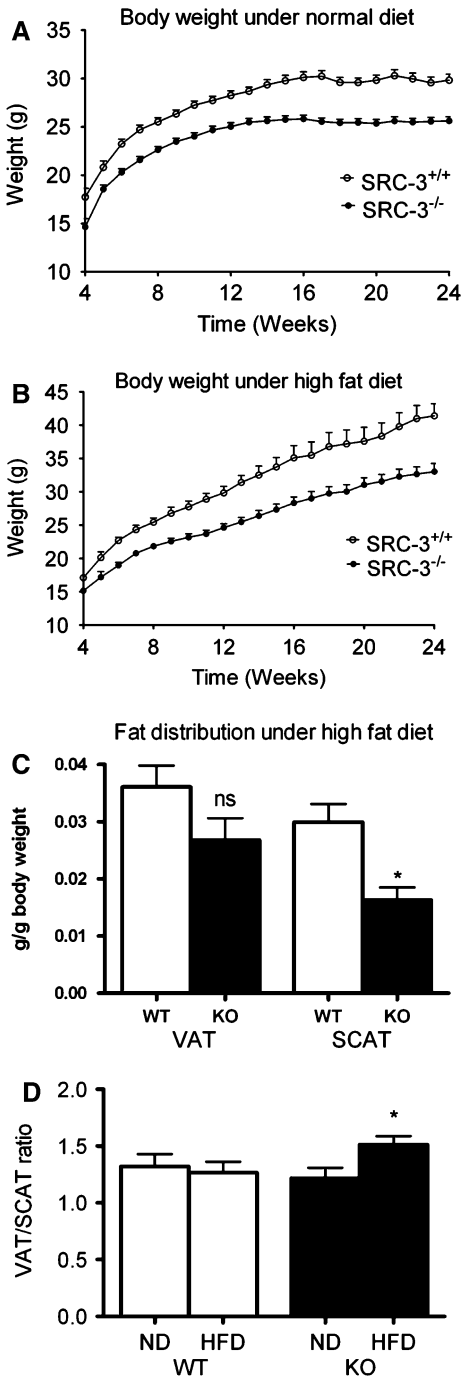

Fig. 1 Onset of diet induced obesity in wild-type and SRC-3$^{-/-}$ mice under HFD. **a** Body weight evolution of mice on normal diet (ND, $n = 23$). **b** Body weight evolution of mice on high-fat diet (HFD, $n = 14$). **c** Average visceral (VAT) and subcutaneous (SCAT) fat weights (normalized by total body weights) in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice under HFD ($n = 14$). **d** Weight ratio between visceral and subcutaneous fat (VAT/SCAT ratio) in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice under both ND and HFD (ND, $n = 14$; HFD, $n = 14$). All data are mean ± SEM. * $P < 0.05$, WT versus KO

As shown in Fig. 1c, under HFD, SRC-3$^{-/-}$ mice had significantly decreased SCAT and marginally decreased (but not significant) VAT compared to that of SRC-3$^{+/+}$ mice. The weight ratio between VAT and SCAT (VAT/SCAT ratio) in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice under ND and HFD was then examined (Fig. 1d). We discovered that under both diet conditions, SRC-3$^{+/+}$ mice had similar VAT/SCAT ratio, indicating that the adipose tissues accumulated proportionally in the VAT and subcutaneous area in SRC-3$^{+/+}$ mice under HFD. However, in SRC-3$^{-/-}$ mice, the ratio between VAT and SCAT increased significantly in HFD compared to ND, suggesting that under energy challenge, the adipose tissues accumulated preferentially in the VAT area of SRC-3$^{-/-}$ mice. These data suggested that under HFD, fat distributed differently between SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice. Compared to the balanced fat deposition in SRC-3$^{+/+}$ mice, in SRC-3$^{-/-}$ mice, energy challenge-induced adipose tissue expansion seemed to be occurred preferentially in the VAT area and thus causing fat redistribution in SRC-3$^{-/-}$ mice under HFD.

Decreased adiponectin and increased corticosterone levels in SRC-3$^{-/-}$ mice

To further confirm that SRC-3$^{-/-}$ mice developed fat redistribution with more VAT than SCAT under HFD, we measured the serum levels of adiponectin and corticosterone in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice. Circulating level of adiponectin, a novel adipokine, has been reported to be negatively correlated with the degree of VAT adiposity in human subjects [20, 21]. Meanwhile, the increased corticosterone levels play a pivotal role in the body fat redistribution in Cushing's syndrome and metabolic syndrome, which both include VAT obesity as one of their major diagnostic features [22, 23]. Consistent with the reported references, we observed a significant decrease in adiponectin concentration (Fig. 2a) and an apparent elevation in corticosterone level (Fig. 2b) in the serum of SRC-3$^{-/-}$ mice compared to SRC-3$^{+/+}$ mice. These serological data further confirmed our observation that SRC-3$^{-/-}$ mice developed fat redistribution and accumulated more VAT than SCAT under HFD.

Different adipogenesis in VAT and subcutaneous adipocytes of SRC-3$^{-/-}$ mice

We then set to tackle the inner mechanism governs the body fat redistribution in SRC-3$^{-/-}$ mice under HFD. It is reported that in the absence of SRC-3, PPARγ gene expression is blunted, which in turn causes an impairment of white adipocyte differentiation in SRC-3$^{-/-}$ mice [10]. Figure 3a and b confirmed this result, as PPARγ expression

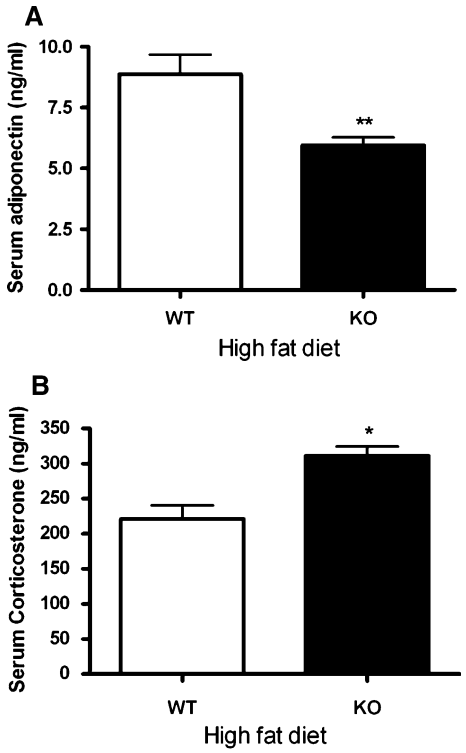

Fig. 2 Serum adiponectin (**a**) and corticosterone (**b**) levels of SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice under HFD ($n = 14$). All data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$, WT versus KO

in both VAT and SCAT decreased significantly in SRC-3$^{-/-}$ mice compared to SRC-3$^{+/+}$ mice under HFD. However, when comparison of gene expression was made between VAT and SCAT, we surprisingly discovered that in SRC-3$^{-/-}$ mice, expression levels of adipocyte marker adipocyte lipid-binding protein 2 (aP2/ALBP) and PPARγ were significantly elevated in VAT versus SCAT, while SRC-3$^{+/+}$ mice displayed similar expression levels of these genes in both fat pads. PPARγ protein expression levels confirmed our results on mRNA levels (Fig. 3c, d). These data indicated that although overall adipogenesis is impaired in SRC-3 deficiency, VAT adipocytes obtained greater differentiation capability than subcutaneous adipocytes in SRC-3$^{-/-}$ mice under HFD, leading to more mature adipocytes and thus larger fat pad in VAT versus SCAT.

Distinct thermogenic and lipolytic genes expression in VAT and SCAT of SRC-3$^{-/-}$ mice

Besides differentiation ability of adipocytes, the size of fat pads is also tightly regulated by energy expenditure. It is thus possible that the preferential deposition of adipose tissue in the VAT area compared to subcutaneous area of SRC-3$^{-/-}$ mice might be caused by the different thermogenic and lipolytic abilities of VAT and subcutaneous

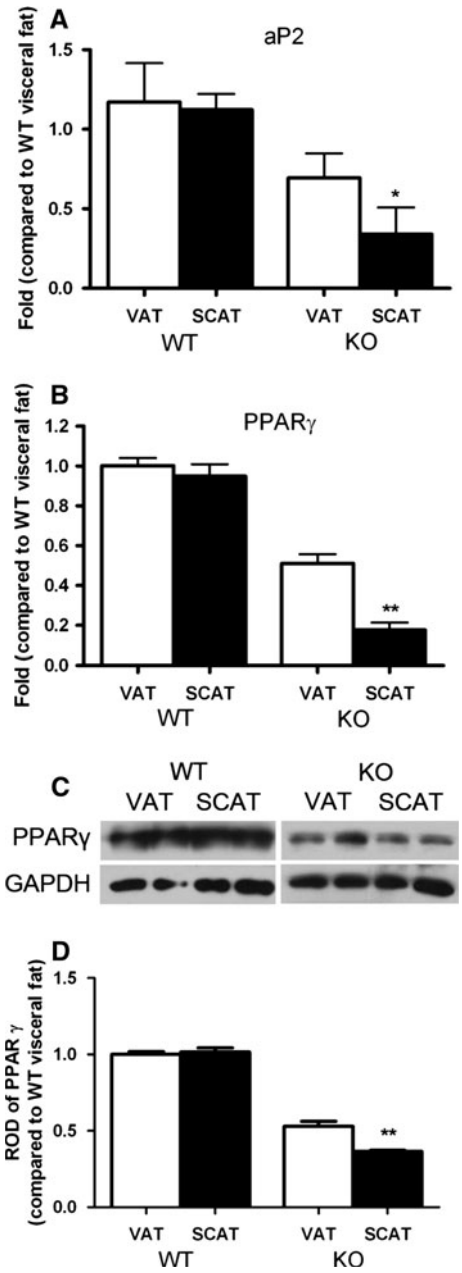

Fig. 3 Different adipogenesis in visceral and subcutaneous adipocytes of HFD SRC-3$^{-/-}$ mice. **a**, **b** Adipogenic marker genes aP2 and PPARγ mRNA levels in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice ($n = 14$). **c** PPARγ protein levels in VAT and SCAT of SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice under HFD ($n = 3$). GAPDH was used as an invariant control. **d** Relative optical density of PPARγ expression. All data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$, VAT versus SCAT in SRC-3$^{-/-}$ mice

adipocytes. Therefore, we tested the expression levels of master genes in thermogenic and lipolytic pathway in VAT and SCAT of SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice. As shown in Fig. 4a and b, in SCR-3$^{-/-}$ mice, uncoupling protein-1 (UCP-1) and PPARγ coactivator-1α (PGC-1α) expression levels both showed a moderate elevation tendency in SCAT versus VAT, while the expression were similar in VAT and SCAT of SRC-3$^{+/+}$ mice. Meanwhile, expressions of genes critical to lipolytic rate, hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), were significantly increased in the SCAT compared to VAT in SRC-3$^{-/-}$ mice, while both genes expressed similarly in SRC-3$^{+/+}$ VAT and SCAT (Fig. 4c, d). These results indicated that, when compared to SRC-3$^{-/-}$ VAT, adipocytes in SRC-3$^{-/-}$ SCAT had enhanced thermogenic and lipolytic abilities, causing more fat utilized as fuel for heat and free fatty acid production and thus less fat accumulated in SCAT than VAT under HFD.

Discussion

Previous studies have shown that SRC-3$^{-/-}$ mice had significantly less VAT under ND due to impaired white adipogenesis and were protected against HFD induced obesity resulted from enhanced energy expenditure [10, 11]. It is thus concluded that SRC-3 played a key role in white adipogenic program and energy expenditure. However, these preliminary researches did not address the question of the different impact of SRC-3 deficiency on VAT and SCAT since adipocytes types, their endocrine function, lipolytic activity, response to insulin, and other hormones differ significantly between fat present in VAT area and subcutaneous area [24]. Therefore, we examined fat distribution in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice in HFD by calculating the weight ratio between VAT and SCAT (VAT/SCAT ratio) and comparing them with VAT/SCAT ratio in both genotypes under ND. Strikingly, we reported here for the first time that mice lacking SRC-3 developed fat redistribution under HFD. Compared to the similar VAT/SCAT ratio in SRC-3$^{+/+}$ mice in both ND and HFD, SRC-3$^{-/-}$ mice showed significantly higher VAT/SCAT ratio in HFD versus ND, suggesting SRC-3$^{-/-}$ mice accumulated more VAT than SCAT under HFD. These results are of particular importance with the increasing clinical recognition that obesity is a heterogeneous disorder and that metabolic profile and degree of associated cardiovascular and metabolic risk varies greatly within obese individuals with different body fat distribution [25, 26].

We then set to reveal the inner mechanism of the differential influence of SRC-3 deficiency on VAT and SCAT. SRC-3$^{-/-}$ mice had been demonstrated to have impaired adipocyte differentiation in VAT under ND [10]. However, it seemed that under HFD, loss of SRC-3 might have greater inhibition on adipogenesis in SCAT than in VAT, as revealed by the elevation of PPARγ and aP2 expression in SRC-3$^{-/-}$ VAT versus SCAT. It is reported that SRC-3 acts synergistically with C/EBP to control gene expression of PPARγ [10]. Besides SRC-3, various

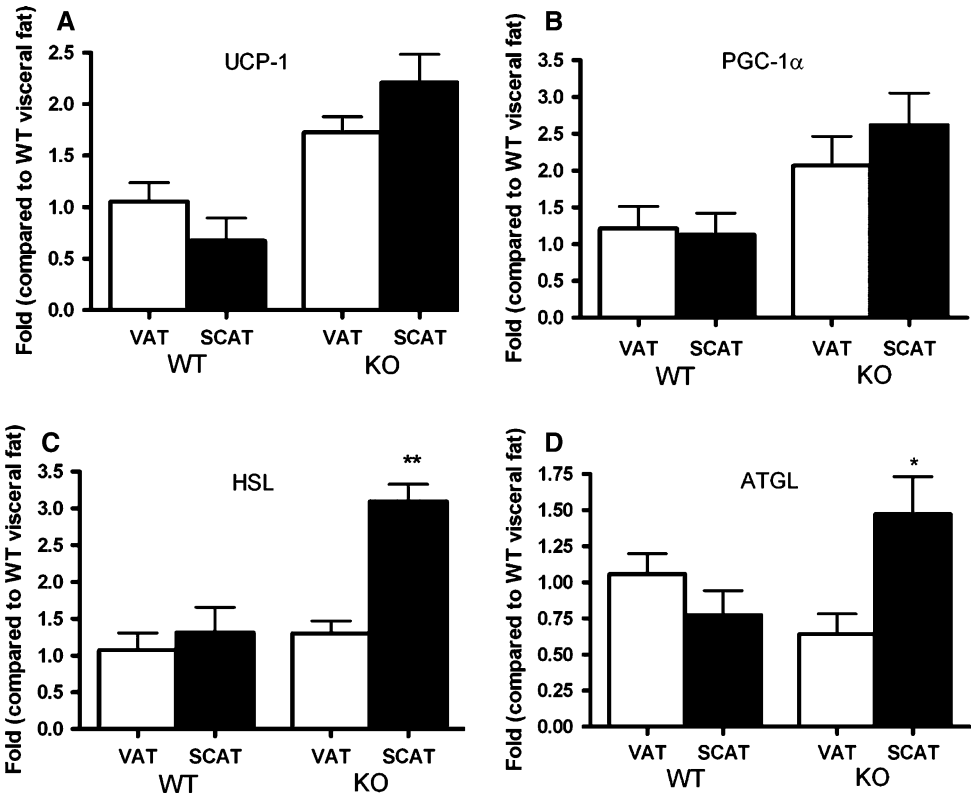

Fig. 4 Distinct thermogenic and lipolytic genes expression in VAT and SCAT of SRC-3$^{-/-}$ mice. **a**, **b** Thermogenic marker genes UCP-1 and PGC-1α mRNA levels in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice ($n = 14$). **c**, **d** Lipolytic genes HSL and ATGL levels in SRC-3$^{+/+}$ and SRC-3$^{-/-}$ mice ($n = 14$). All data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$, VAT versus SCAT in SRC-3$^{-/-}$ mice

cofactors and transcription factors are also involved in the strict and complex regulating network of PPARγ expression [27]. Since adipocytes from VAT and SCAT are molecularly different with each other [24], the difference in SRC-3 deficiency on PPARγ inhibition in VAT and SCAT could be attributed to the different levels of cofactors/transcription factors concentration in these two fat deposits.

Functional hypercortisolism in patients with metabolic syndrome and Cushing's syndrome is suggested to be pathogenically involved in the induction of VAT obesity, the common characteristics of both diseases [28]. We noticed a significant increase in circulation cortisone level in SRC-3$^{-/-}$ mice compared to SRC-3$^{+/+}$ mice, which could contribute to the fat redistribution in HFD SRC-3$^{-/-}$ mice. Adipocytes from VAT and SCAT are different in the density and binding affinity of glucocorticoid receptors. Glucocorticoid receptors in VAT adipocytes have higher concentration and more substrate binding than that in subcutaneous adipocytes, leading to enhanced preadipocyte maturation and lipid formation in VAT [29]. Therefore, SRC-3 deficiency could lead to prolonged exposure of SRC-3$^{-/-}$ mice to excess cortisone and result in a preferentially increased fat accumulation in SRC-3$^{-/-}$ VAT.

Thermogenic genes expression induced by HFD in white adipose tissue has been associated with obesity resistance in rodents [30]. In SRC-3$^{-/-}$ mice, we found a significantly increased expression of PGC-1α and UCP1, which confirmed the conclusion that SRC-3$^{-/-}$ mice were protected against obesity under energy challenge due to enhanced energy expenditure rate [11, 12]. Surprisingly, we also found a greater (but not statistically significant) induction of PGC-1α and UCP1 in SCAT versus VAT in HFD SRC-3$^{-/-}$ mice compared to a slightly decrease in HFD SRC-3$^{+/+}$ mice. Furthermore, expression of two enzymes critical to lipolytic rate, HSL and ATGL, all enhanced significantly in SCAT of HFD SRC-3$^{-/-}$ mice, while they had similar even lower expression in SCAT of HFD SRC-3$^{+/+}$ mice compared to that in VAT. Therefore, SRC-3$^{-/-}$ mice could counter SCAT accumulation under HFD through the specific elevation in expression of genes involved in thermogenic and lipolytic pathways.

We observed significantly decreased circulating adiponectin level along with regional VAT expansion in SRC-3$^{-/-}$ mice. Similar phenomenon is wildly presented in clinical patients with central obesity and significantly decreased serum adiponectin levels [21, 31]. This negative correlation between adiponectin and VAT content was explained by the fact that adipocytes from VAT are major site for adiponectin secretion upon insulin stimulation and that VAT from patients suffered central obesity have impaired adiponectin produce ability due to loss of insulin sensitivity [32]. It is thus possible that in HFD SRC-3$^{-/-}$ mice, adiponectin reduction was caused by decreased secretion from VAT due to attenuated insulin sensitivity

compared to SCAT. This postulation is not contradictory to previous results which showed SRC-$3^{-/-}$ mice had improved insulin profile than SRC-$3^{+/+}$ mice under HFD, since the enhanced insulin sensitivity could be contributed mainly by SCAT of SRC-$3^{-/-}$ mice.

It should be noted that compared to 11β-HSD-1 knockout mice, which exhibited decreased VAT and improved insulin sensitivity under HFD, SRC-$3^{-/-}$ mice in our study presented increased VAT but a more favorable metabolic profile. This contradiction could be explained by the fact that SRC-$3^{-/-}$ mice were intrinsically obesity resistance and did not develop extensive obese under energy challenge [10, 11]. The average body weight of SRC-$3^{-/-}$ mice after the duration of HFD administration is approximately 33 g (Fig. 1b), only slightly exceeding the average body weight of age-matched SRC-$3^{+/+}$ mice under ND (around 30 g, Fig. 1a). Therefore, although SRC-$3^{-/-}$ mice had proportionally more VAT after HFD, they were not obesity models and did not display the adverse effect of VAT obesity on metabolic parameters.

In conclusion, we demonstrated in vivo results that SRC-3 deficiency exerted different impact on VAT and SCAT accumulation. Under HFD, SRC-$3^{-/-}$ mice developed fat redistribution. HFD SRC-$3^{-/-}$ mice accumulated more VAT than SCAT and had significantly higher VAT/SCAT ratio than age-matched ND SRC-$3^{-/-}$ mice, while SRC-$3^{+/+}$ mice showed similar regional fat distribution and VAT/SCAT ratio under both ND and HFD. A specific enhancement in adipogenesis in VAT and an elevation in lipolysis and thermogenesis-related gene expression in SCAT of HFD SRC-$3^{-/-}$ mice along with increased corticosterone level in serum of SRC-$3^{-/-}$ mice could be possible explanations to the fat redistribution occurred in SRC-$3^{-/-}$ mice under HFD. Since some clinical disorders, i.e., Cushing's syndrome, HIV-associated adipose redistribution syndrome and metabolic syndrome [33, 34], are characterized with VAT obesity, the primary pathogenic cause of which is a preferential VAT expansion in patients, our results might provide novel clues to the pathogenesis and therapeutic researches on these diseases.

Materials and methods

Animal experiment

SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice were kindly given by Baylor College of Medicine. All animals were maintained in a hybrid C57BL/6J × 129/Sv background (93.75%/6.25%, respectively) and housed in an environment with a controlled temperature of 22°C and 12 h alternating darkness and light cycles. Only male and age-matched mice were used for the experiments. Animals were given free access to water and normal (carbohydrate 60%; protein 22%; fat 10%; cellulose and other nutrient factors 8%) or HFD (Research Diets, D12492, 20 kcal% carbohydrate; 20 kcal% protein; 60 kcal% fat). Mice were anaesthetized and killed by exsanguinations. VAT and SCAT were dissected, weighed, and partitioned for follow-up experiments. Animal protocols were approved by the Institutional Animal Care and Use Committee.

ELISA

Blood samples were collected by retro-orbital bleeds on anaesthetized mice. For serum preparation, blood samples were centrifuged at 3000 rounds per minute (rpm) for 10 min at 4 C. The clear upper layers containing serums were then carefully transferred to new tubes. Mice serum adiponectin and corticosterone levels were measured using adiponectin ELISA kit (Linco) and the corticosterone radioimmunoassay kit (ICN Pharmaceuticals) following manufacturer's instruction.

RNA extraction and first strand cDNA synthesis

Approximately 100 mg VAT and SCAT were excised from SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice ($n = 14$). All specimens used for quantitative PCR were snapped frozen upon extraction and stored at −80°C until use. Total RNA was isolated from tissue samples using TRIZOL reagent (Invitrogen, CA) following the manufacturer's instructions. One microgram of total RNA was converted into first strand cDNA with the First Strand cDNA Synthesis kit (Promega, USA) according to the protocol recommended by the manufacturer.

Quantitative PCR

Quantitative PCR was performed using Light Cycler Fast Start DNA Master SYBR Green I (TAKARA, Japan). The sequences of all used primers are available upon request. PCR array data were calculated by the ΔΔCt method and normalized against house keeping gene β-actin.

Western blotting

Approximately 100 mg tissue was isolated from each mouse. Samples were kept in a polypropylene snap-cap tube containing Complete Lysis-M, EDTA-free (Roche, USA) and homogenized on ice for 30 s. The homogenized samples were kept on ice for 10 min and centrifuged at 10,000 rpm for 10 min at 4 C. Then, the clear upper layers were transferred to new tubes and assayed for protein concentration by Dc protein assay kit (Bio-Rad, USA). Lysates from VAT and SCAT of SRC-$3^{+/+}$ and SRC-$3^{-/-}$ mice were separated on

SDS-PAGE, transferred to nitrocellulose and blotted with primary antibodies directed against mouse anti-PPAR $\gamma 2/\gamma 1$ monoclonal antibody (Chemicon, USA) and GAPDH (as loading control, Cell signaling, USA) followed by secondary antibody (anti-mouse IgG horseradish peroxidase-linked antibody, Cell Signaling, USA) and chemiluminescent detection. Relative optical density (ROD) of each band was quantified and calculated by BioRad-image for Windows Program (BioRad GS-800 Calibrated Densitometer).

Statistics

Animal experiments were performed with between 6 and 10 animals per experimental group. Results were expressed as mean ± SEM. Comparisons between groups were performed using *t*-test (GraphPad Prism version 4.03). Differences were considered significant if $P < 0.05$.

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