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Ablation of Adipose-HO-1 Expression Increases White Fat over Beige Fat Through Inhibition of Mitochondrial Fusion and of PGC1alpha in Female Mice

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Original Article

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Ablation of adipose-HO-1 expression increases white fat over beige fat through inhibition of mitochondrial fusion and of PGC1α in female mice

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Abstract:
Background: Hmox1 plays an important role in the regulation of mitochondrial bioenergetics and function by regulating cellular heme-derived CO and bilirubin. Previous studies have demonstrated that global disruption of HO-1 in humans and mice resulted in severe organ dysfunction.
Methods: We investigated the potential role of adipose-specific-HO-1 genetic ablation on adipose tissue function, mitochondrial quality control and energy expenditure by generating an adipo-HO-1 knockout mouse model (Adipo-HO-$1^{-/-}$) and, in vitro, adipocyte cells in which HO activity was inhibited. Adiposity, signaling proteins, fasting glucose and oxygen consumption were determined and compared to adipocyte cultures with depressed levels of both HO-1/HO-2.
Results: Adipo-HO-$1^{-/-}$ female mice exhibited increased adipocyte size, and decreases in the mitochondrial fusion to fission ratio, PGC1, and SIRT3. Importantly, ablation of HO-1 in adipose tissue resulted in fat acquiring many properties of visceral fat such as decreases in thermogenic genes including pAMPK and PRDM16. Deletion of HO-1 in mouse adipose tissue led to complete metabolic dysfunction, an increase in white adipose tissue, a reduction of beige fat and associated increases in FAS, aP2 and hyperglycemia. Mechanistically, genetic deletion of HO-1 in adipose tissues decreased the mitochondrial fusion to fission ratio; disrupted the activity of the PGC1 transcriptional axis and thermogenic genes both in vitro and in vivo.
Conclusion: Ablation of adipose tissue-HO-1 abridged PGC1 expression promoted mitochondrial dysfunction and contributed to an increase of pro-inflammatory visceral fat and abrogated beige-cell like phenotype.

Keywords: bilirubin, CO, heme oxygenase, Mnf2, PGC1α
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Introduction

Obesity is considered a major risk factor for the development of vascular dysfunction, inflammation, insulin resistance and metabolic syndrome. Chronic obesity and an increase of visceral fat is associated with adipocyte dysfunction that contributes to an increase in reactive oxygen species (ROS) and changes in adipocyte-derived paracrine factors [1], [2], [3]. Obesity-mediated development of hyperglycemia suppresses HO-1 levels [4], [5], [6]. Hyperglycemia is associated with an increase in ROS and peroxynitrite that results [7], [8], [9] in an increase in cellular heme levels [7], [9], [10]. Increased heme levels are essential to increase adipocyte terminal differentiation and adipogenesis in vivo [10], [11]. However, an excessive increase in heme in conjunction with a decrease in HO-1 function may lead the adipocyte to proceed to terminal differentiation and inflammation [12], [13]. An increase in heme and a reduction in HO-1 levels, as seen in HO-1 deletion, results in a detrimental

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cellular effect due to increased ROS levels triggered by heme and H_2O_2, with as a consequence an increase in the inflammatory properties of both monocytes and macrophages [14], [15] and increased adipocyte dysfunction [16], [17], [18].

Studies in humans and mice show that lack of HO-1 causes rupture of macrophages and tissue inflammation due to exposure of non-metabolized heme released on erythrophagocytosis [19]. The discovery that HO-1 may be a novel target for modulation of the inflammatory response [20], [21] and diminished fibrosis [22], has increased interest in HO-1 signaling pathways.

Adipose tissue function is controlled by several processes including mitochondrial biogenesis, adaptive thermogenesis, mitochondrial fatty acid oxidation, oxygen consumption and oxidative phosphorylation that are regulated by PGC-1α [23], [24], [25]. Adipocytes store fat and excess energy, however, beige and brown adipocytes are regarded as the major source of mitochondrial and thermogenic function to combat obesity and metabolic syndrome [23], [26]. Several transcriptional factors regulate the levels of signaling molecules that are involved in the expression of thermogenic and mitochondrial function in beige and visceral fat [23], [25], [26], [27], [28], [29].

Transduction of HO-1 in mice fed a high fat diet mitigated weight gain and decreased visceral fat content. Higher levels of HO-1 increased the number of adipocytes of small cell type, which is described as "browning of the white fat" (or thermogenic fat). This thermogenic fat is a unique phenotype called beige fat and is distinct from both white fat and brown fat, increasing adiponectin and sonic hedgehog and decreasing inflammatory cytokines [16].

Administration of an EET agonist, HO-1 inducer inhibited terminal differentiation and inflamed adipogenesis, decreased cytokine levels and increased the number of small cell adipocytes or beige cells [17]. Likewise, PCG-1α expression is induced by exposure to cold [30], [31]. PCG-1α is a major regulator of mitochondrial biogenesis and oxidative metabolic pathways and induces mitochondrial and thermogenic genes such as UCP-1. Similarly, ablating PGC1-α results in reduced capacity for adaptive thermogenesis through activation of beige fat cells when exposed to cold [32]. Together, this demonstrates that PGC-1α plays an important role in beige fat cell development and function [33]. Additionally, there is a mitochondrial network function that depends on signaling molecules and the relationship between mitochondrial fusion and fission. While mitochondrial fission is orchestrated by the dynamin-related protein 1 (DRP1) and the mitochondrial fission 1 (Fis1) protein [34], [35], the fusion process is controlled by the autosomal dominant optic atrophy 1 (OPA1) protein, together with the mitochondrial fusion proteins mitofusion 1 and 2 (Mfn 1 and 2), located on the mitochondrial outer membrane, [36], [37]. As HO-1/HO-2 are known regulators of mitochondrial integrity and function [38], [39], [40], [41], we hypothesized that adipose HO-1 gene is essential for increased mitochondrial fusion that may result in an increase in the beige cell population within adipose tissue or conversion of white adipose function populations and white adipose function that include expression of PGC1α levels and thermogenic genes.

Materials and methods

Cell culture and treatment

Pre-adipocytes; 3T3-L1 were maintained and cultured in adipogenic medium as described [12]. Cells were treated with the HO activity inhibitor, SnMP (2 μM, Tin mesoporphyrin), every 3-days and harvested as described [12].

Animals

Adipocyte specific HO-1 null mice were generated by breeding AdipoQ^{Cre} and Hmox1$^{fl/fl}$ mice on a C57BL/6 genetic background. Mice homozygous for floxed Hmox1 carry at least one allele for cre recombinase under the adiponectin promoter were used. Subsequently the generated mice were knockout for HO-1 in adipose tissue. Mice were fed a normal chow diet for 30 weeks and matched with age-matched wild type C57BL/6 mice. In this study only female mice were used.

Lentiviral vectors under the control of the adipocyte-specific promoter aP2 were constructed using the Lenti-Max™ system (Lentigen, Baltimore, MA, USA). Lentiviruses (50 μL, 2×10^9 TU/mL in saline) were injected into the littermate of aP2-HO-1$^{-/-}$ mice by a single intracardiac injection. Two weeks later a second injection was completed (75 μL 1×10^9 TU/mL in tail vein) as previously described [16]. Mice were divided into two groups (n = 6 per group): adipo-HO-1$^{+/+}$ and adipo-HO-1$^{-/-}$ lenti-aP2-HO-1 at 30 weeks of age. Mice were weighed every week, blood glucose was determined [16], [18], [42]. All experimental protocols were performed follow-

ing an IACUC of New York Medical College and a animal protocols were approved by the Institutional Animal Care and the University of Mississippi Medical Center approved protocol in accordance with the *NIH Guide for the Care and Use of Laboratory Animals*.

RNA/real-time polymerase chain reaction (PCR) of thermogenic and mitochondrial genes

Total RNA was extracted from 3T3 cells using TRIzol® (Ambion, Austin, TX, USA) and from frozen adipose tissues by RNeasy® Lipid Tissue (Qiagen), as per instructions provided by the manufacturers. Specific TaqMan® Gene Expression Assays probes for mouse HO-1, PGC1α, COX-IV (cytochrome c oxidase subunit-IV), adiponectin, TNFα, and other signaling RNA were determined as previously described [42], [43].

Western blot analysis

Frozen mouse adipose tissues were ground under liquid nitrogen and suspended in homogenization buffer (mmol/L: 10 phosphate buffer, 250 sucrose, 1.0 EDTA, 0.1 PMSF, and 0.1% v/v tergitol, pH 7.5). For in vitro Western blot analysis pelleted cells were lysed and HO-1 and other signaling proteins were measured [12], [44].

Morphological adipose tissue evaluation

Adipose tissue was prepared for morphological analysis. Samples were cut using a microtome (5 μm thick), mounted on D-polylisinated glass slides, deparaffinizated in xylene and either stained with hematoxylin and eosin for the evaluation of adipocyte size [16].

Statistical analysis

Data are expressed as means ± standard error of mean (SEM). Significance of difference in mean values was determined using one-way analysis of variance followed by the Newman-Keul's post hoc test. $p < 0.05$ was considered to be significant.

Results

HO activity inhibition reduces PGC1α, and mitochondrial signaling in cell culture

Western blot data demonstrate significant ($p < 0.05$) inhibition of PGC1α protein levels with SnMP-treated cells compared to WT cells. Furthermore, protein expression of SIRT1 and SIRT3 was significantly ($p < 0.05$) decreased in the SnMP treated cells as compared to WT cells (Figure 1A–D).

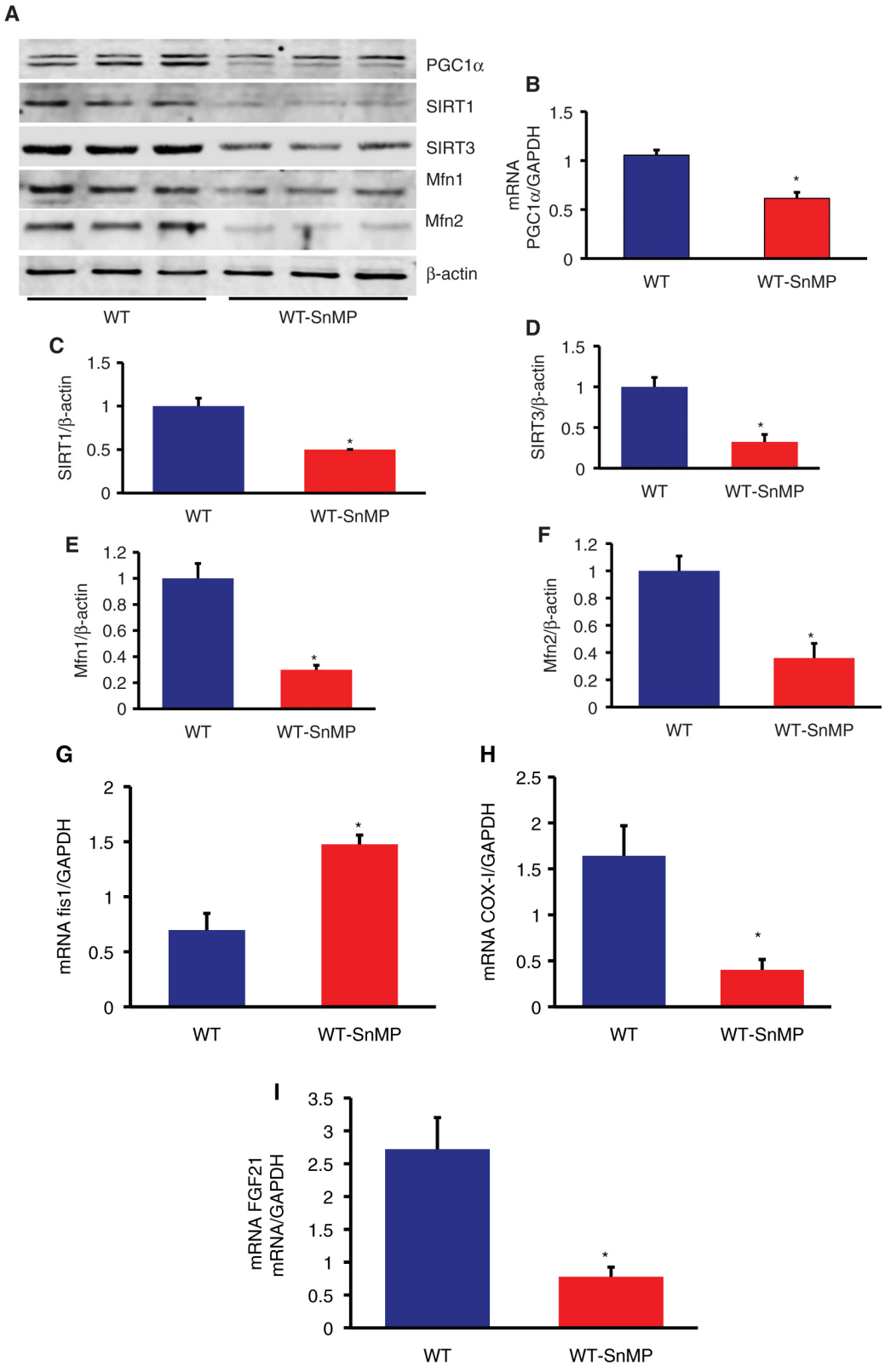

Figure 1: Effect of inhibition of HO activity by SnMP on WT and 3T3-L1 adipocytes cells on gene expression related to mitochondrial biogenesis and dynamics.
(A) Representative Western blots of PGC1α, SIRT1, SIRT3, Mfn1and Mfn2 proteins. Densitometric analyses of (B) PGC1α, (C) SIRT1, (D) SIRT3, (E) Mfn1 and (F) Mfn2 proteins. mRNA expression of (G) Fis1, (H) COX-I and (I) FGF21 in WT and 3T3-L1-derived adipocyte cells treated with SnMP. Results are mean ± SE, n = 4, *p < 0.05 vs. WT.

To examine whether inhibition of HO activity can modulate the mitochondrial fusion-to-fission ratio we treated adipocytes with SnMP. RT-PCR data show increased mRNA expression levels of fission related Fis1 as compared to WT cells ($p < 0.05$) (Figure 1G). Interestingly the mitofusion related Mfn1 and Mfn2 protein expression levels were significantly ($p < 0.05$) decreased in WT cells treated with SnMP as compared to WT

cells alone (Figure 1A, E and F). The mRNA expression levels of COX-I and FGF21 were significantly ($p < 0.05$) decreased in the SnMP treated cells as compared to WT cells. (Figure 1H and I).

HO activity inhibition increases expression of adipogenic markers in cell culture

SnMP increased ($p < 0.05$) the expression of the adipogenic markers Rev-Erb α, PPARγ and aP2 (Figure 2). Western blot analysis demonstrated that SnMP increased the expression levels of Rev-Erbα as compared to WT cells (Figure 2A and B). Similarly mRNA expression of adipogenic PPARγ and aP2 significantly ($p < 0.05$) increased in adipocyte cells as compared to WT cells (Figure 2C and D) ($p < 0.05$).

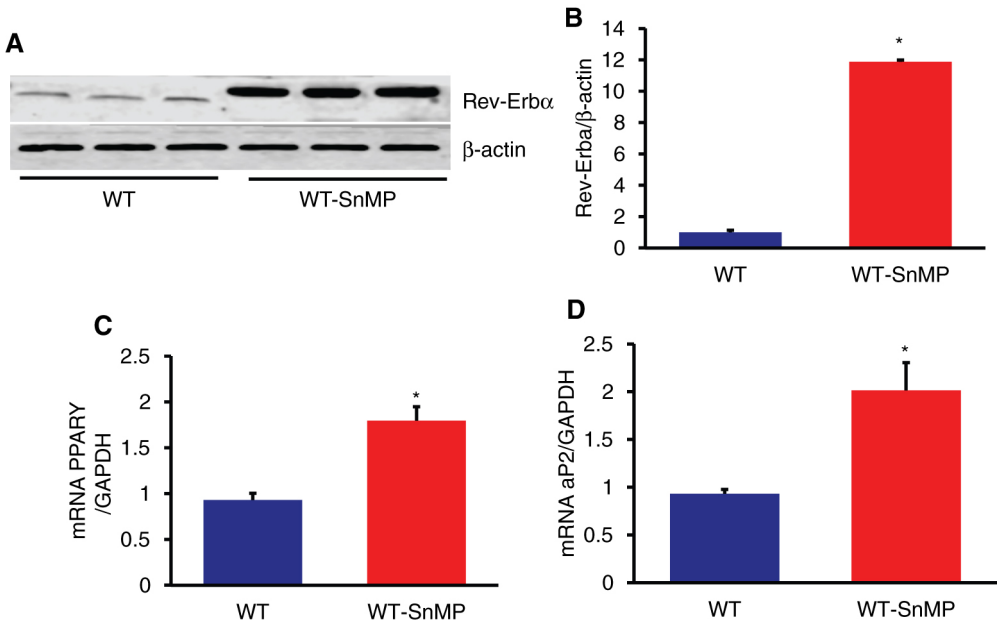

Figure 2: Effect of inhibition of HO activity by SnMP on WT and 3T3-L1 adipocytes cells on adipogenic Rev-Erbα, PPARγ and aP2 expression.
(A) Representative Western blots of Rev-Erbα proteins. Densitometric analyses of (B) Rev-Erbα proteins. mRNA expression of (C) PPARγ and (D) aP2 in WT and 3T3-L1-derived adipocyte cells treated with SnMP. Results are mean ± SE, n = 4, *$p < 0.05$ vs. WT.

HO-1 genetic deletion is a negative regulator and white fat expression and body weight in female mice

We created a mouse strain with the specific deletion of the HO-1 gene using the cre-lox system. At birth, the Adipo-HO-$1^{-/-}$ mice appeared normal and were indistinguishable from their control littermates. At 30 weeks of age Adipo-HO-$1^{-/-}$ mice exhibited an 11% increase in body weight compared with age-matched WT mice ($p < 0.05$) Figure 3A. By 30 weeks of age Adipo-HO-$1^{-/-}$ mice exhibited increases in epididymal (0.40 ± 0.06 vs. 0.74 ± 0.09; $p < 0.05$) and visceral fat (0.35 ± 0.04 vs. 0.6 ± 0.07; $p < 0.05$), suggesting that the increases in fat may be critical in Adipo-HO-1-null adiposity (Figure 3B and C). Fasting blood glucose levels in HO-1-null mice were 120.6 ± 2.55 mg/dL compared with 99.5 ± 2.08 mg/dL in WT mice ($p < 0.05$) (Figure 3D).

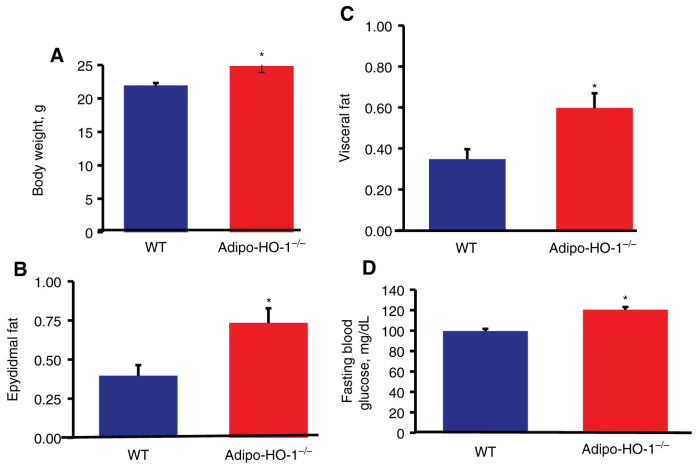

Figure 3: (A) Body weight of 30-week-old female WT and adipocyte specific HO-1 knockout mice (B) epydidmal fat, and (C) visceral fat content in mice fed a normal diet (wild-type [WT] and adipocyte specific knock out (KO) (D) fasting blood glucose in mice fed a normal diet WT and KO. n = 4–6; *p < 0.05 vs. WT.

Expression of RNA and signaling protein in Adipo-HO-1$^{-/-}$ female mice

Adipo-HO-1$^{-/-}$ mice display decreases in HO-1 at the levels of both mRNA and protein ($p < 0.001$) (Figure 4A, B, and C). To determine whether adipocyte specific knockout affected HO-2 levels, we performed real time (RT)-PCR analysis for HO-2 RNA levels which clearly demonstrated that HO-1 deletion was not associated with an increase in HO-2 expression when compared to WT mice (Figure 4D).

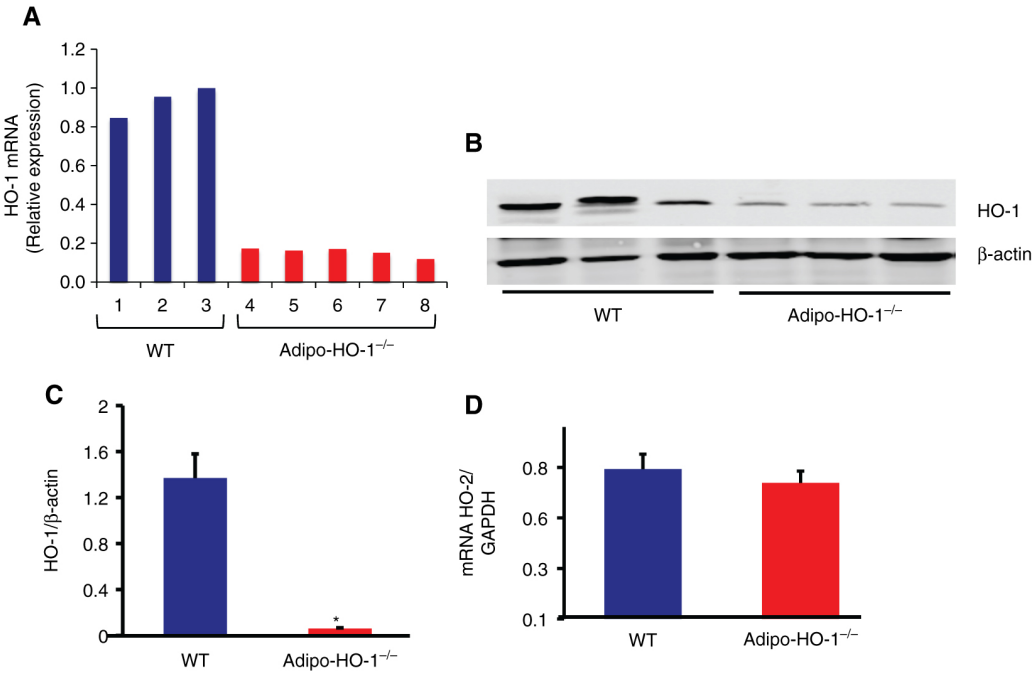

Figure 4: RT-PCR and Western blots analyses of adipose tissue of mice: (A) relative HO-1 mRNA expression with individual WT as well as in KO mice, (B) WBs showed protein expression of HO-1 with adipose tissue in WT and KO mice, (C) HO-1 WBs densitometry analyses in WT and KO mice, (D) relative HO-2 mRNA expression, (E) adipocytes size, (F) measurements of adipocytes size in adipose tissue of WT and KO mice; n = 4–6, *p < 0.05 vs. WT.

Adipo-HO-1 gene deletion decreases oxygen consumption in female mice

We examined the effect of adipocyte specific deletion of HO-1 on both O_2 consumption and the ratio of CO_2/O_2 in mice. As expected, HO-$1^{-/-}$ mice displayed a decrease in VO_2 consumption ($p < 0.05$) (Figure 5A). However, control animals had a significant ($p < 0.05$) increase in oxygen consumption with a concomitant lowering of VCO_2/VO_2 (Figure 5A and B). Importantly protein expression of COX-IV was significantly ($p < 0.05$) decreased in the HO-1 knockout mice as compared to WT mice (Figure 5C and D).

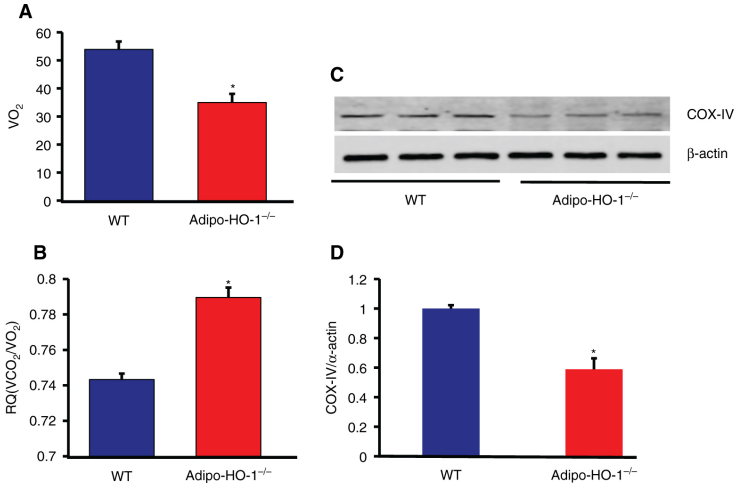

Figure 5: Effect of HO-1 deletion on oxygen consumption and respiratory quotient (RQ).
(A) Mouse respiratory oxygen consumption, (B) respiratory quotient (RQ). Representative Western blots of COX-IV proteins (C) and (D) densitometric analyses of COX-IV in adipose tissue of WT and KO mice; n = 4–6, *p < 0.05 vs. WT.

Adipo-HO-1 gene deletion reduces thermogenic mitochondrial fusion and fission genes in female mice

Western blot analysis clearly demonstrated a decrease ($p < 0.05$) in the protein expression of PGC-1α in Adipo-HO-$1^{-/-}$ mice compared with those in age-matched WT mice (Figure 6A and B). SIRT1 was significantly ($p < 0.05$) decreased in HO-1-null mice at both the mRNA and protein expression levels compared to WT mice (Figure 6A and C). SIRT3 expression was decreased with HO-1 ablation ($p < 0.05$), similarly SIRT1 decreased with HO-1 deletion in adipose tissue of mice ($p < 0.05$) (Figure 6A and D).

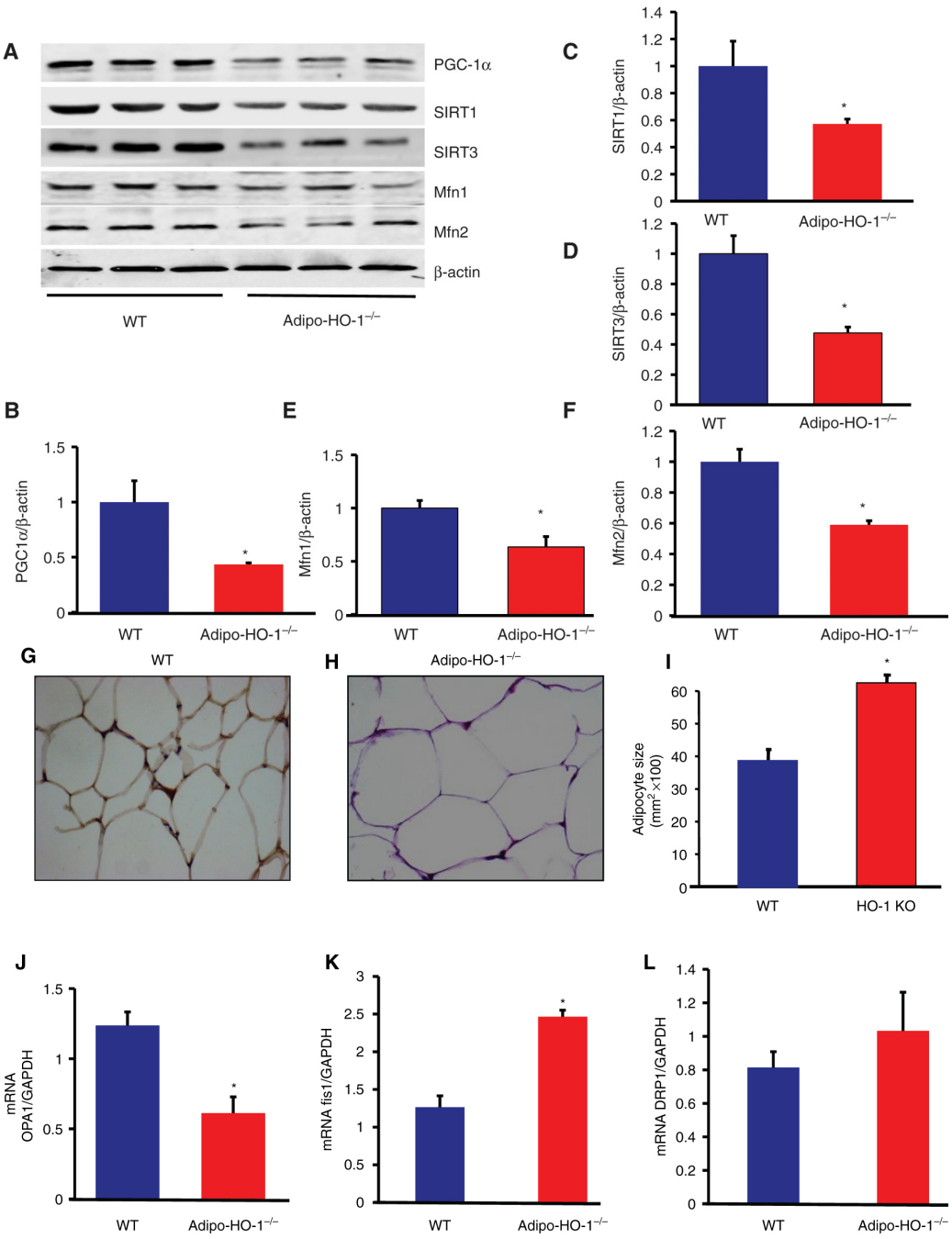

Figure 6: Effect of HO-1 deletion on PGC1α, SIRT1, SIRT3, Mfn1 and Mfn2 proteins. (A) Representative Western blots of PGC1α, SIRT1, SIRT3, Mfn1and Mfn2 proteins. Densitometric analyses of (B) PGC1α, (C) SIRT1, (D) SIRT3, (E) Mfn1 and (F) Mfn2 proteins. Hematoxylin-eosin staining depicts adipocyte size of WT (G), and (H) adipo-HO-1 null, (I) adipocyte count. RT-PCR analyses represents mRNA expression of (J) OPA1, (K) Fis1 and (L) DRP1 in mice in adipose tissue of WT and KO mice; n = 4, *p < 0.05 vs. WT.

The expression of mitochondrial fusion related to proteins in HO-1 null mice was significantly ($p < 0.05$) reduced as compared to WT mice during WB analysis. The adipose tissue of HO-1 null mice exhibited significantly ($p < 0.05$) decreased of mitofusion related Mfn1 and Mfn2 protein expression as well as mRNA expression (Figure 6A, E and F). We examined the adipocyte size by hematoxylin-eosin in WT and Adipo-HO-$1^{-/-}$ in mice adipose tissue. HO-1 null mice showed significant ($p < 0.05$) increase in adipocyte size compared to WT mice (Figure 6G, H and I).

However, RT-PCR results demonstrated that there was significantly increased of mitofission related Fis1 mRNA expression levels (Figure 6K). The fusion related OPA 1 mRNA was significantly ($p < 0.05$) reduced in HO-1 null mice (Figure 6J). There was no effect on DRP1 mRNA expression (Figure 6L).

Effect of Adipo-HO1-Null on AMPK and signaling in adipose tissues

The protein content and phosphorylation of AMPK were reduced ($p < 0.05$) in adipose tissue from Adipose HO-1 knockout mice compared to WT (Figure 7A and B). Densitometry analysis did not show a significant decrease in the expression of phosphorylation insulin receptors of Adipo-HO-1-null mice as compared to WT mice (Figure 7A, C and D). mRNA levels of adiponectin in Adipo-HO-1-null mice were significantly ($p < 0.05$) lower than those in age-matched WT mice (Figure 7E). PRDM16 is involved in the development and function of classical brown and beige adipocytes. Our study found a significant decrease ($p < 0.05$) in PRDM16 mRNA expression in Adipo-HO-1-null mice as compared to WT mice (Figure 7F).

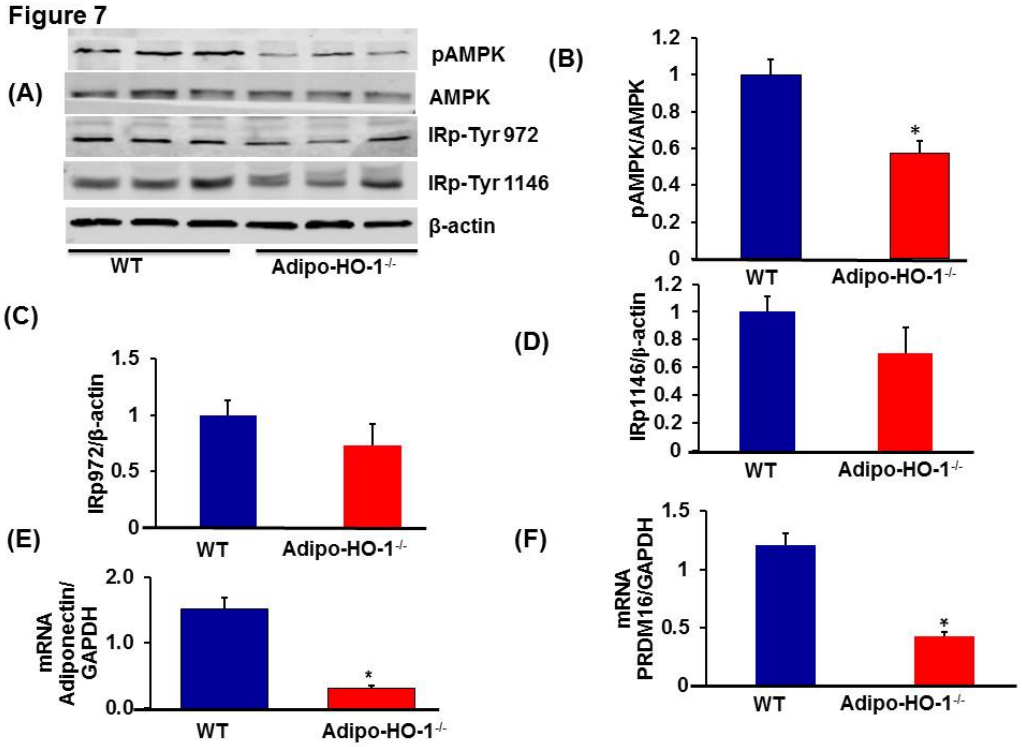

Figure 7: (A) Western blots and densitometry analyses of (B) pAMPK, (C) pAKT, (D) IRp972 and (E) IRp1146. mRNA expression of (E) adiponectin and (F) PRDM16 in adipose tissue of WT and KO mice; *$p < 0.05$ vs. WT.

Adipo-HO-1$^{-/-}$ mice display increased expression of adipogenic and inflammatory markers

We showed a significant increase ($p < 0.05$) in Rev-Erbα expression in Adipo-HO-1-null mice compared to WT control mice (Figure 8A and B). Further, Adipo-HO-1 deletion results in a significant increase in the protein expression of FAS ($p < 0.05$) compared with age-matched WT mice (Figure 8A and C). Similarly, Adipo-HO-1-deletion was associated with an increase in the expression of adipogenic aP2 protein levels ($p < 0.05$) (Figure 8A and D).

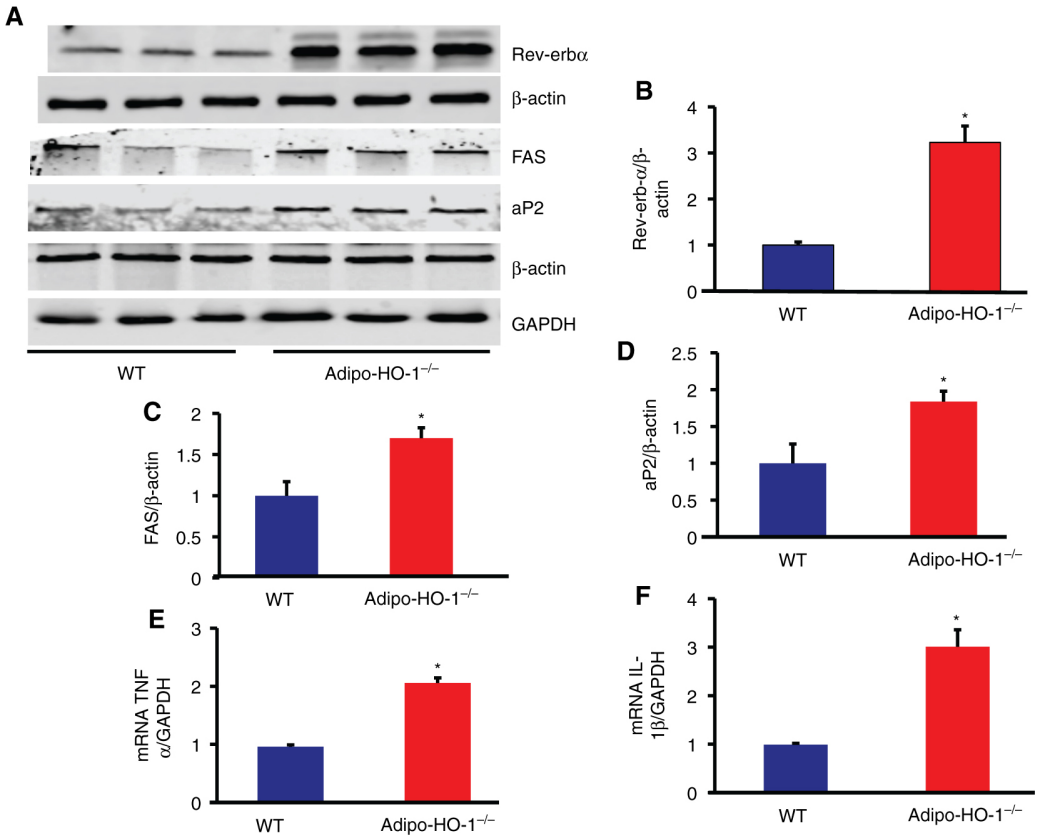

Figure 8: (A) Western blots and densitometry analyses of (B) Rev-Erbα, (C) FAS, (C) aP2 and mRNA expression of (E) TNFα and (F) IL-6 in adipose tissue of WT and KO mice; *p < 0.05 vs. WT.

Obesity is frequently accompanied by systemic inflammation and this was examined in the current study by measuring proinflammatory markers relative mRNA expression levels. The HO-1-null mice had increased ($p < 0.05$) levels of inflammatory cytokines in adipose tissue compared with age-matched WT mice seen in (Figure 8E and F). Our study reports significant increases ($p < 0.05$) in TNFα and IL1β mRNA expression in Adipo-HO-1-null mice as compared to WT mice (Figure 8E and F).

Adipose tissues specific of HO-1 using lentiviral ap2 promoter rescues Adipo-HO-1$^{-/-}$ phenotype in female mice

We examined adipocyte size in both HO-1$^{-/-}$ mice and in WT littermates using hematoxylin-eosin and compared adipo-HO-1$^{-/-}$ transfected with lenti-aP2-HO-1 mice (Figure 9). Adipo-HO-1$^{-/-}$ mice displayed an increase ($p < 0.05$) in adipocyte cell size (hypertrophy) compared to WT mice adipocyte (Figure 9A, B and D). Adipocyte hypertrophy seen in Adipo-HO-1$^{-/-}$ were rescued by administration of lenti-aP2- HO-1 viral vector (Figure 9C and D). Further, signaling of mitochondrial and thermogenic genes in Adipo-HO-1$^{-/-}$ was restored to WT adipose levels following transfection with lenti-aP2-HO-1. Our study found a significant decrease ($p < 0.05$) in PRDM16 mRNA expression in Adipo-HO-1-null mice as compared to WT mice, which was reversed by administration of lenti-aP2- HO-1 viral vector (Figure 9E). Similarly the adipose tissue of HO-1 null mice exhibited significantly ($p < 0.05$) decreased levels of HO-1, PGC-1α and mitofusion related Mfn1 and Mfn2 protein expression and which were rescued by administration of lenti-aP2- HO-1 viral vector (Figure 9F–J).

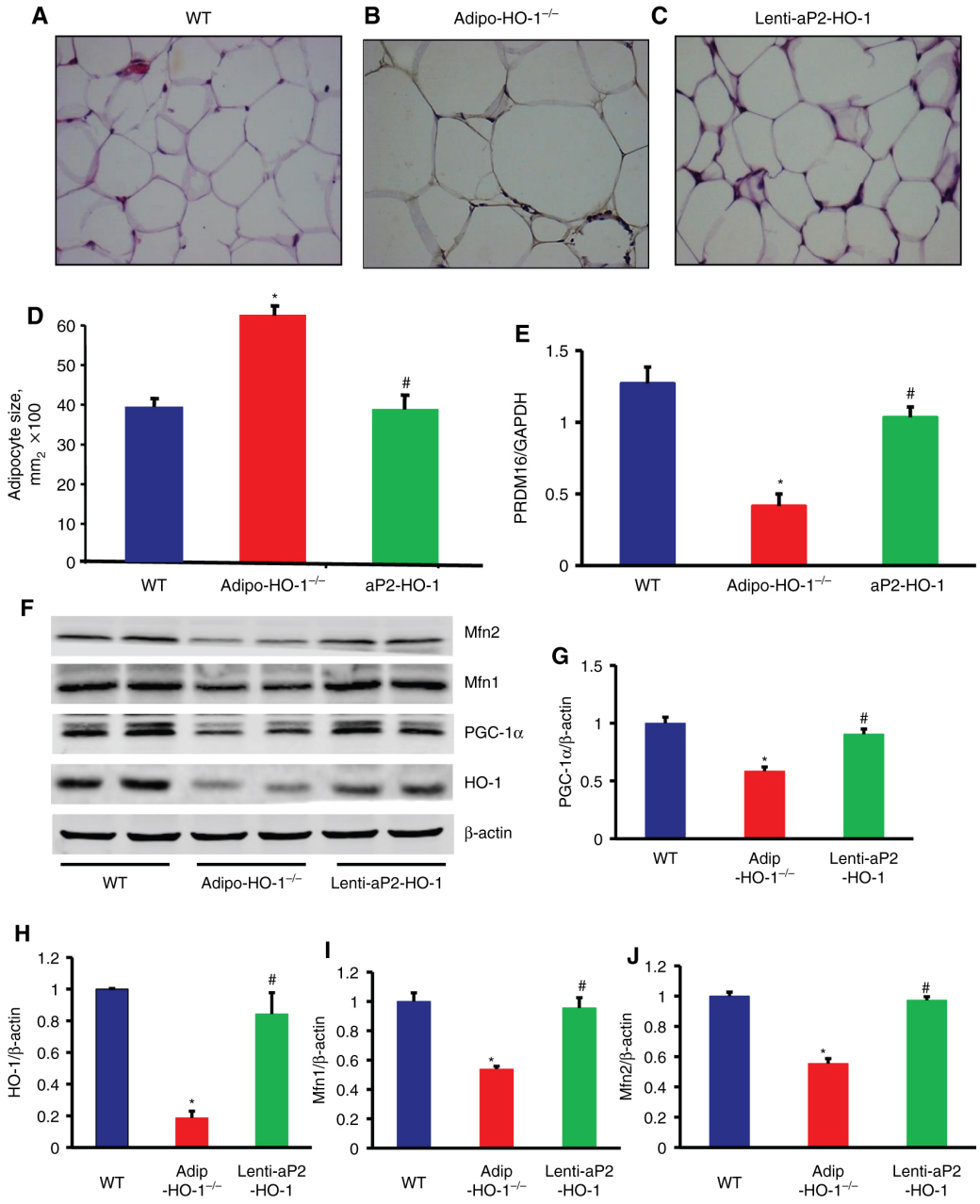

Figure 9: Hematoxylin-eosin depicts adipocyte size of WT (A), adipo-HO-1null (B) and (C) adipo-HO-1 over expressed (D) adipocyte measurements in adipose tissue of WT, KO and HO-1 overexpressed mice; n = 10–15. (E) mRNA expression of PRDM16, (F) Western blots and densitometry analyses of (G) PGC1α, (H) HO-1, (I) Mfn1, (J) Mfn2 in adipose tissue of WT, KO and HO-1 overexpressed mice; n = 4, *p < 0.05 vs. WT, #p < 0.05 vs. KO.

Discussion

In the present report, we constructed a mouse model with the specific ablation of adipose HO-1 that affect visceral fat expansion and inflammation. As a result, adipose specific HO-$1^{-/-}$ animals exhibited a significant reduction in mitochondrial integrity genes, thermogenic gene expression and O_2 consumption. Adipo-HO-$1^{-/-}$ developed enlargement of visceral fat and adipocyte hypertrophy that was associated with hyperglycemia. Importantly, deletion of HO-1 resulted in a marked decrease in PGC-1α levels and important genes that belong to beige and brown adipocytes [30], [45]. Reduction of PGC-1α levels is associated with insulin resistance and hyperglycemia [46] and a decrease of mitochondrial oxidation phosphorylation and metabolic regulators [45], [47]. PGC-1α gene expression enhanced the changes to brown-like fat from white fat [30]. Additionally, Adipo-HO-$1^{-/-}$ displays a reduction of PRDM16, a major player in browning adipose tissue. These results are in agreement with the findings that the ablation of PRMD16 in mice increased visceral fat and inhibited ther-

mogenic genes [48]. PRDM16 is necessary for brown fat phenotype and is an important factor in the conversion of WAT to beige fat under beta adrenergic stimulation [49].

PRDM16 activates the expression of thermogenic and mitochondrial genes [49]. This allows the beige fat to have increased mitochondrial content and uncoupled respiration, resulting in thermogenesis.

WAT can be transformed into thermogenic adipose tissue or "beige" fat. Both classic thermogenic tissue (BAT) and inducible thermogenic tissue (beige fat) increase heat production through an uncoupling oxidative metabolism from ATP production [50]. The leak in the proton gradient caused by the UCP-1 means that fuel oxidation can be accelerated and is not limited by saturation concentration of ATP and causes heat production [51]. Similarly, levels of AMPK were decreased on Adipo-HO-$1^{-/-}$ mice. AMPK regulation of mitochondrial function and white to beige like cells [29] and reprograms adipocyte cells differentiation to healthy adipocytes to release adiponectin [16], [44], [52].

Our data showed that the adipocyte HO-$1^{-/-}$ phenotype can be rescued by lentiviral-aP2-HO-1, with the reversal of visceral fat adipocyte size to smaller healthy adipocytes. We previously showed that targeting adipose tissue using adipocyte tissue specific HO-1 (aP2-HO-1) decreases pro-inflammatory adipokines and adipocyte cell size that produces adiponectin, i.e. beige-like cells [16]. Our present data shows that HO-1 deletion abridged mitochondrial dynamics, biogenesis in female mice by the downregulation of mitochondrial fusion over fission and increased adipocyte hypertrophy.

Essentially balanced mitochondrial dynamics is important for the maintenance of mitochondrial health, function and energy generation [39], [53]. Concomitantly inhibition of HO activity reduces mitochondrial quality control by inhibition of mitochondrial fusion mediator Mfn1, Mfn2 and Opa1 but activation of mitochondrial fission mediator Fis1 [42], [43]. Hence, our study offers a portal on the unique role of HO-1 in adipose tissue by its ability to maintain mitochondrial quality and biogenesis. HO-1 expression appears to affect adipocyte function through the regulation of heme bioavailability. This comprises heme containing denatured proteins, generation of the bioactive metabolites carbon monoxide and biliverdin, inhibiting cellular buildup of free heme, and preventing free radical accumulation and mitochondrial dysfunction [2], [54]. Recently, we demonstrated that EET-agaonist-mediated HO-1 induction ameliorates progressiono f cardiomyopathy **[58]**.

Spiegelman's group elegantly showed that PGC1α is a regulator of ALA-synthetase, (ALAS) the first and rate limiting enzyme in heme biosynthesis [55]. An increase of heme turnover following an increase of ALAS is associated with a reduction of adiposity, increased mitochondrial function and adipocyte function [56], [57]. These results are in agreement with this report that HO-1 deletion results in a decrease in heme turnover and PGC1α, the latter is essential to restore mitochondrial integrity and increase beige like cells. In contrast, induction of HO-1 increases heme turnover, increases PGC1α, ALAS, while increasing both bilirubin and CO which improve adipose and vascular function via the reduction of ROS and an increase in antioxidant molecules, bilirubin and MnSOD (reviewed in [2], [13]. It can be concluded from this study that HO-1 induction is associated with the reprogramming of adipocytes in white adipose tissue to acquire characteristic of beige fat cell. Consequently, the activation of HO-1 signaling pathway may lead to identification of new therapeutic target that address the metabolic dysfunction associated with the progressive nature of the metabolic syndrome by restoring beige like adipocyte cell population in adipose tissues that can be using for cell therapy (Figure 10).

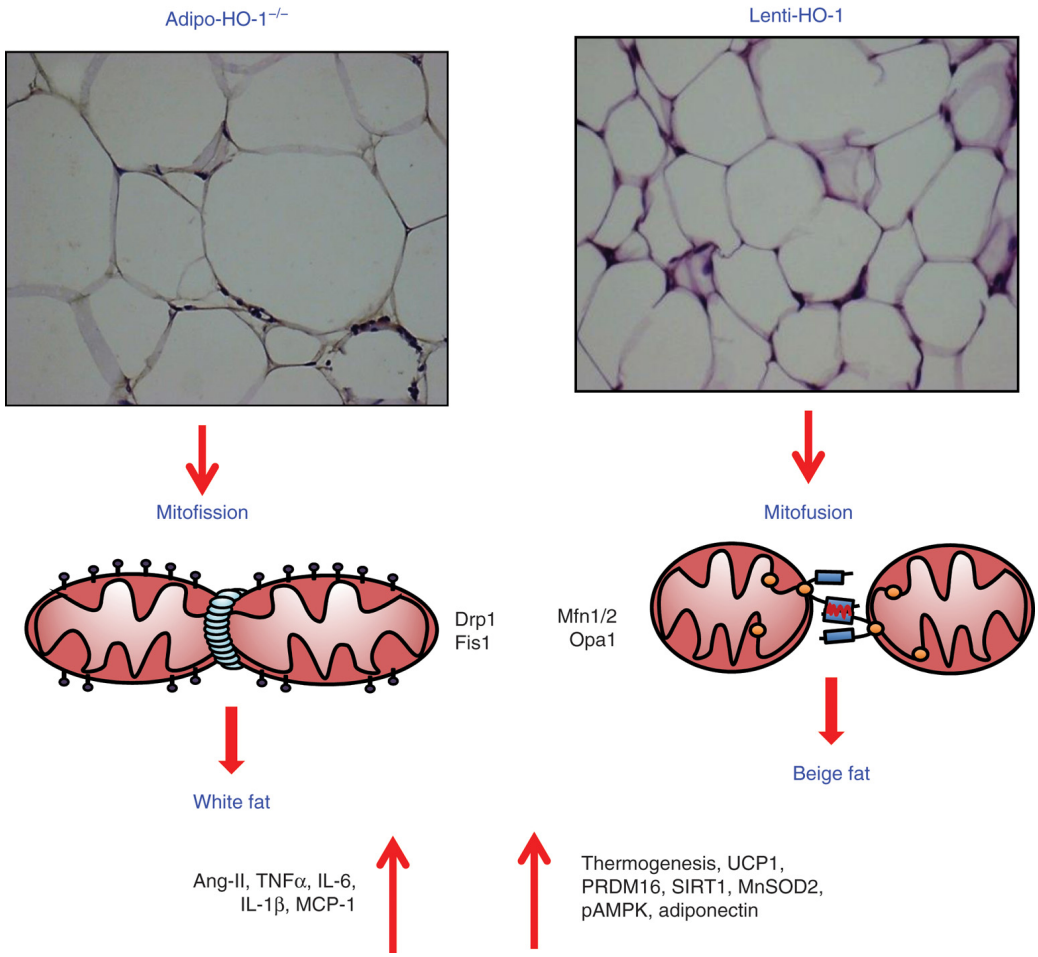

Figure 10: Schematic description showed that HO-1 gene ablation leads to an increase of adipogenic FAS, MEST and aP2 expression, in adipose tissue which is responsible for elevated adipocyte expansion, hypertrophy and releases inflammatory adipokines while decreasing PGC1.
Targeting adipose tissues-specific HO-1 expression increases mitofusion over mitofission in adipocyte culture as well as adipose tissue of mice. It may be concluded that HO-1 is important decreases excessive heme that result in stimulation of thermogenic genes PGC1α, PRDM16 and adiponectin. Over all HO-1 ablation leads to an increase of white fat over beige like phenotype that is prevented by adipo-aP2-HO-1 expression.

In summary, HO-1 deletion abridged mitochondrial biogenesis and function by downregulation of PGCα, SIRT1 Mfn1, Mfn2 and Opa1 but activation of mitochondrial fission mediator Fis1. In contrast lenti-aP2-HO-1 reversed the detrimental effects of HO-1 deletion. HO-1 increased as did HO activity thereby decreasing heme levels and increasing PGC-1α levels with a subsequent decrease in adipogenesis and obesity. The increase in heme turnover enhanced adipocyte differentiation and produced smaller healthy adipocytes [17]. This when considered with the adverse effect of obesity on both HO-1 and PGC-1α in adiposity and the production of healthy beige adipocytes. This study provided valuable insights into therapeutic approaches to control obesity and to insure healthy adipocytes. The importance of the symbiotic relationship between HO-1 and PGC1α also offers a potential therapeutic sight for intervention in the control of obesity and metabolic syndrome to increase beige-fat like population within adipose tissue.

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consent is not applicable. Ethical approval: The research related to animals use complied with all the relevant national regulations and institutional policies for the care and use of animals.

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