

8-1-2017

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

S Singh

I Grant

A Meissner

A Kappas

Nader Abraham

New York Medical College

Follow this and additional works at: https://touro scholar.touro.edu/nymc_fac_pubs



Part of the [Animal Experimentation and Research Commons](#), [Cell Biology Commons](#), and the [Medicine and Health Sciences Commons](#)

Recommended Citation

Singh, S., Grant, I., Meissner, A., Kappas, A., & Abraham, N. (2017). Ablation of Adipose-HO-1 Expression Increases White Fat over Beige Fat Through Inhibition of Mitochondrial Fusion and of PGC1alpha in Female Mice. *Hormone Molecular Biology and Clinical Investigation*, 31 (1). <https://doi.org/10.1515/hmbci-2017-0027>

This Article is brought to you for free and open access by the Faculty at Touro Scholar. It has been accepted for inclusion in NYMC Faculty Publications by an authorized administrator of Touro Scholar. For more information, please contact touro.scholar@touro.edu.

Original Article

Shailendra P. Singh¹ / Ilana Grant² / Aliza Meissner² / Attallah Kappas³ / Nader G. Abraham^{1,4}

Ablation of adipose-HO-1 expression increases white fat over beige fat through inhibition of mitochondrial fusion and of PGC1 α in female mice

¹ Department of Pharmacology, New York Medical College, NY, USA, E-mail: nader_abraham@nymc.edu² Department of Medicine, New York Medical College, NY, USA³ The Rockefeller University, New York, NY 10065, USA, Phone: 212-327-8494, Fax: 212-327-8690, E-mail: kappas@rockefeller.edu⁴ New York Medical College, Valhalla, NY 10595, USA, Phone: +914-594-3121, Fax: +914-347-4956, E-mail: nader_abraham@nymc.edu**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, PGC1 α


DOI: 10.1515/hmbci-2017-0027

Received: May 3, 2017; **Accepted:** June 23, 2017

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

Attallah Kappas, Nader G. Abraham are the corresponding authors.

 ©2017, Attallah Kappas and Nader G. Abraham et al., published by De Gruyter.

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 3.0 License.

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-1 α [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, PGC-1 α expression is induced by exposure to cold [30], [31]. PGC-1 α is a major regulator of mitochondrial biogenesis and oxidative metabolic pathways and induces mitochondrial and thermogenic genes such as UCP-1. Similarly, ablating PGC-1 α results in reduced capacity for adaptive thermogenesis through activation of beige fat cells when exposed to cold [32]. Together, this demonstrates that PGC-1 α 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 PGC1 α 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 μ M, 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 LentiMaxTM system (Lentigen, Baltimore, MA, USA). Lentiviruses (50 μ L, 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 μ L 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, PGC1 α , COX-IV (cytochrome c oxidase subunit-IV), adiponectin, TNF α , 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 μ m thick), mounted on D-polyisinated glass slides, deparaffinized in xylene and either stained with hematoxylin and eosin for the evaluation of adipocyte size [16].

Statistical analysis

Data are expressed as means \pm 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 PGC1 α , and mitochondrial signaling in cell culture

Western blot data demonstrate significant ($p < 0.05$) inhibition of PGC1 α 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).

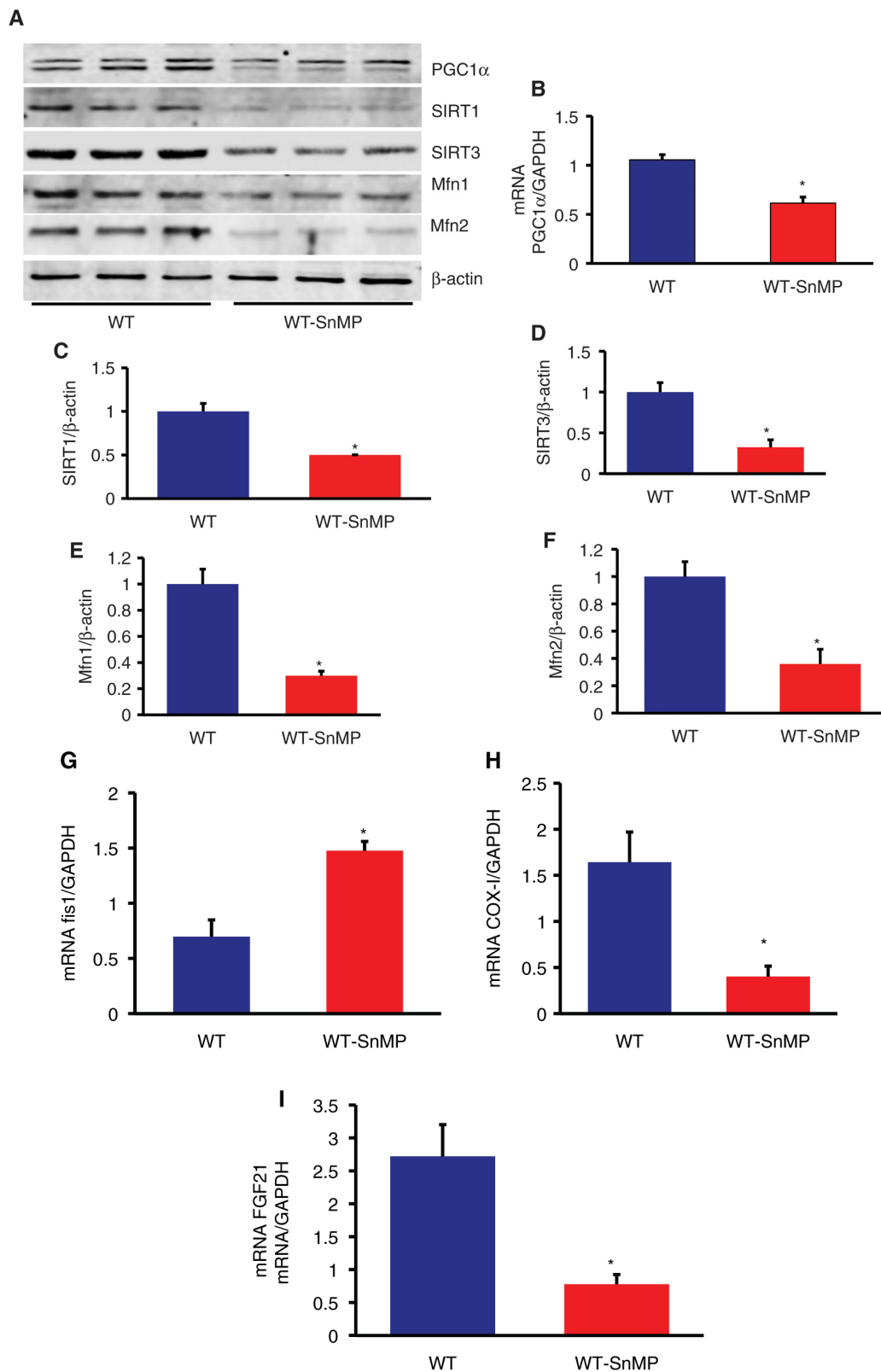


Figure 1: Effect of inhibition of HO activity by SnMP on WT and 3T3-L1 adipocytes cells on gene expression related to mitochondrial biogenesis and dynamics.

(A) Representative Western blots of PGC1 α , SIRT1, SIRT3, Mfn1 and Mfn2 proteins. Densitometric analyses of (B) PGC1 α , (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 \pm 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 , PPAR γ and aP2 (Figure 2). Western blot analysis demonstrated that SnMP increased the expression levels of Rev-Erb α as compared to WT cells (Figure 2A and B). Similarly mRNA expression of adipogenic PPAR γ and aP2 significantly ($p < 0.05$) increased in adipocyte cells as compared to WT cells (Figure 2C and D) ($p < 0.05$).

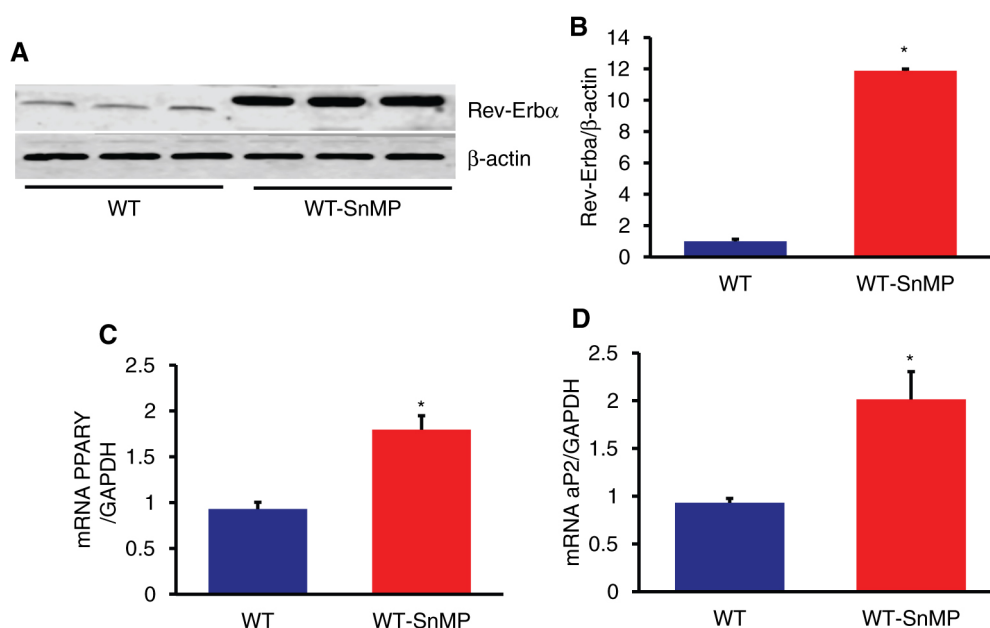


Figure 2: Effect of inhibition of HO activity by SnMP on WT and 3T3-L1 adipocytes cells on adipogenic Rev-Erb α , PPAR γ and aP2 expression.

(A) Representative Western blots of Rev-Erb α proteins. Densitometric analyses of (B) Rev-Erb α proteins. mRNA expression of (C) PPAR γ and (D) aP2 in WT and 3T3-L1-derived adipocyte cells treated with SnMP. Results are mean \pm 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).

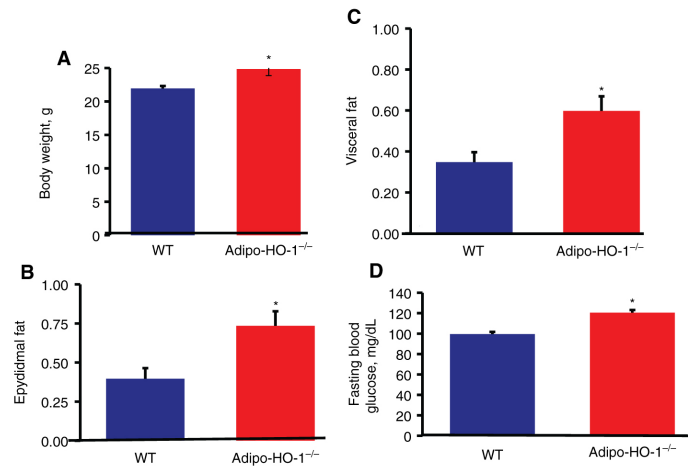


Figure 3: (A) Body weight of 30-week-old female WT and adipocyte specific HO-1 knockout mice (B) epididymal fat, and (C) visceral fat content in mice fed a normal diet (wild-type [WT] and adipocyte specific knock out (KO) (D) fasting blood glucose in mice fed a normal diet WT and KO. $n = 4-6$; * $p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

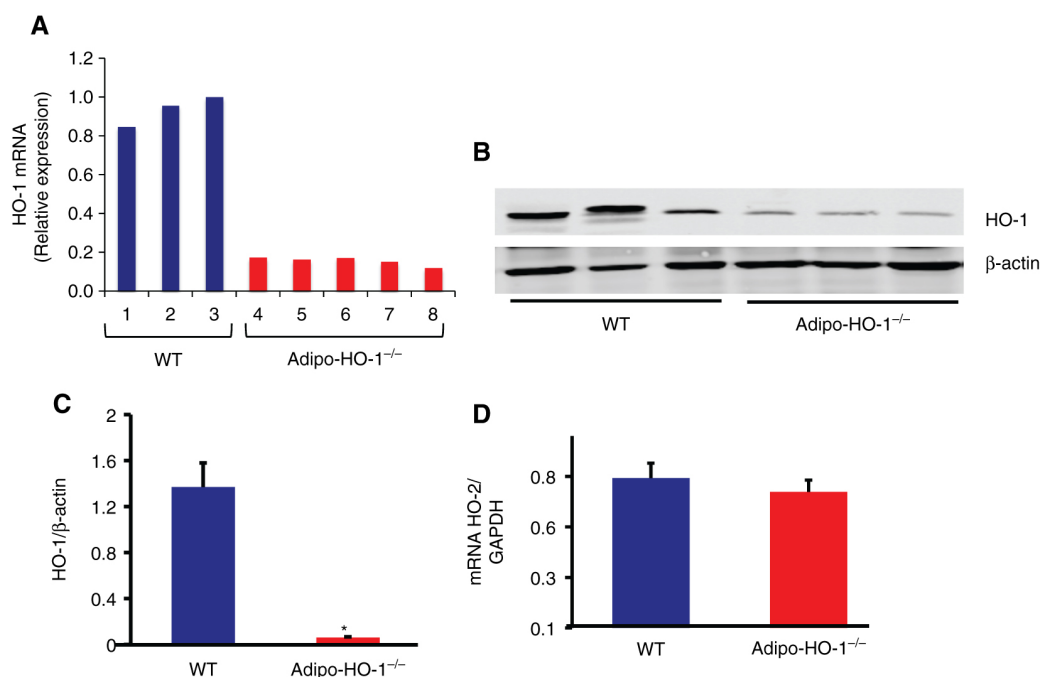


Figure 4: RT-PCR and Western blots analyses of adipose tissue of mice: (A) relative HO-1 mRNA expression with individual WT as well as in KO mice, (B) WBs showed protein expression of HO-1 with adipose tissue in WT and KO mice, (C) HO-1 WBs densitometry analyses in WT and KO mice, (D) relative HO-2 mRNA 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).

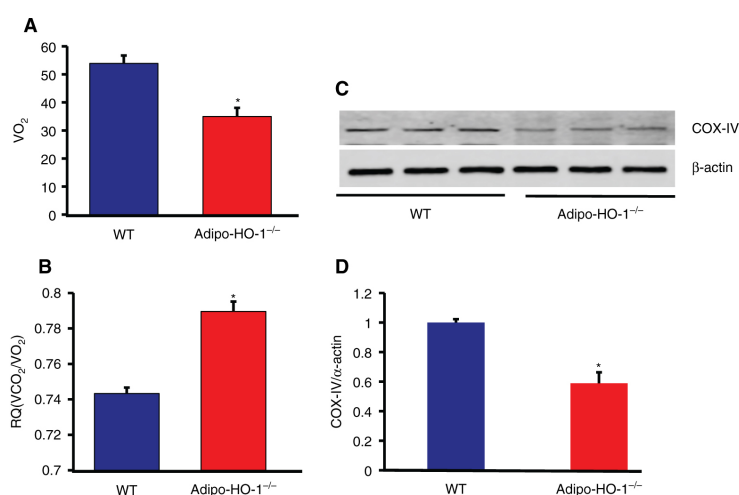


Figure 5: Effect of HO-1 deletion on oxygen consumption and respiratory quotient (RQ).

(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-1 α 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).

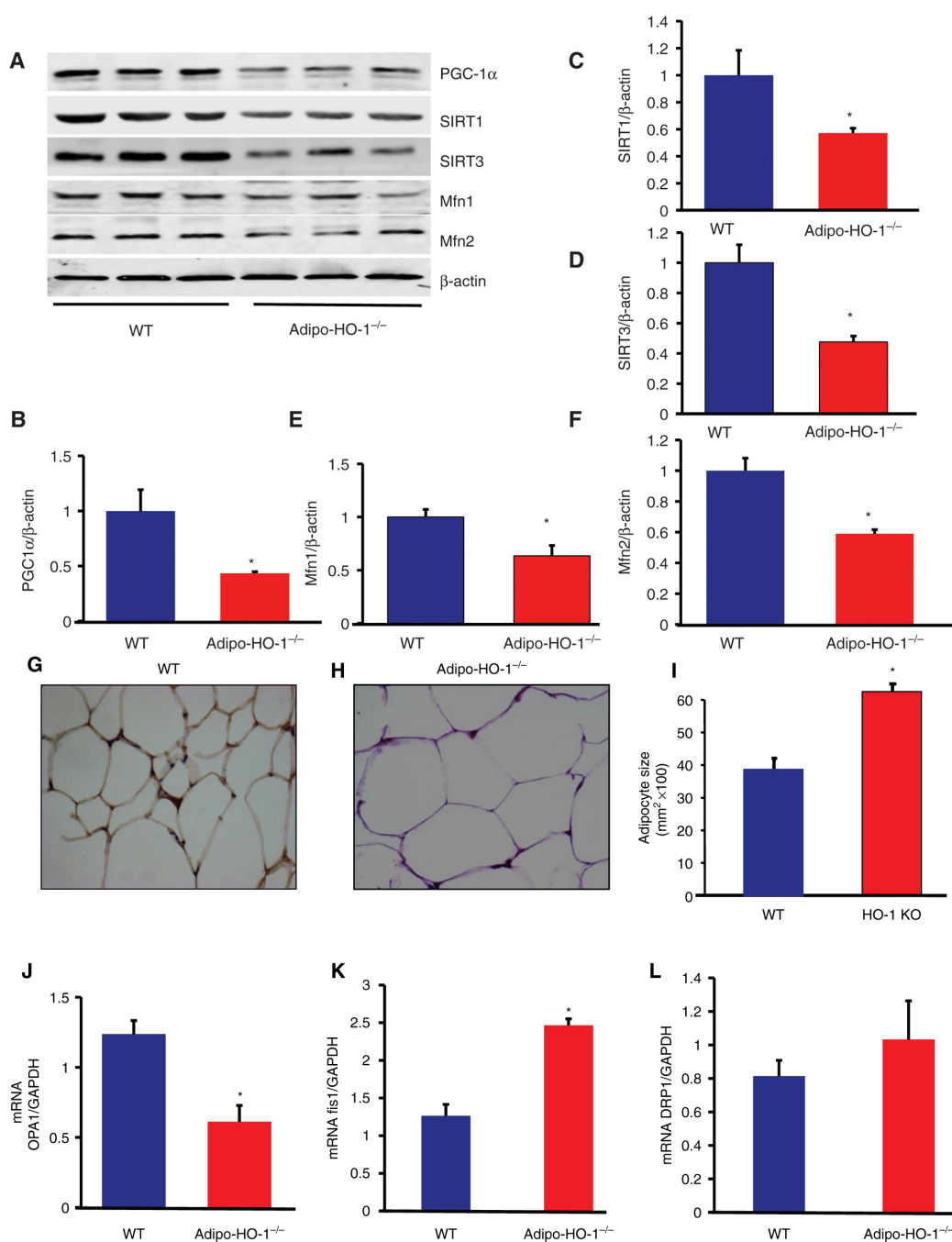


Figure 6: Effect of HO-1 deletion on PGC1 α , SIRT1, SIRT3, Mfn1 and Mfn2 proteins. (A) Representative Western blots of PGC1 α , SIRT1, SIRT3, Mfn1 and Mfn2 proteins. Densitometric analyses of (B) PGC1 α , (C) SIRT1, (D) SIRT3, (E) Mfn1 and (F) Mfn2 proteins. Hematoxylin-eosin staining depicts adipocyte size of WT (G), and (H) 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). 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).

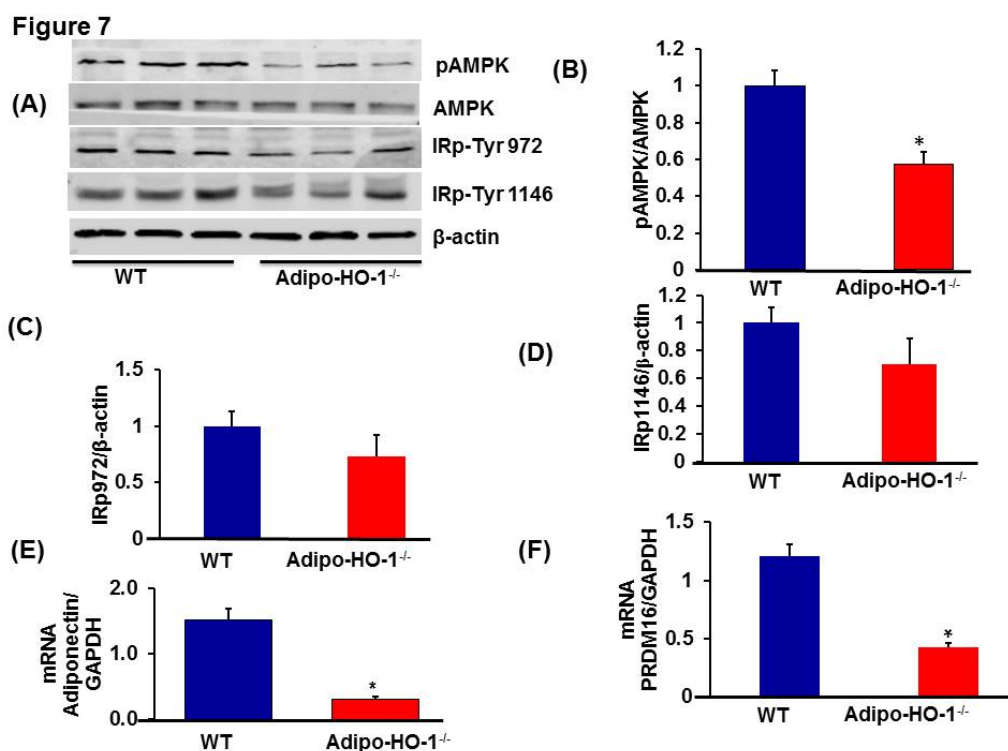


Figure 7: (A) Western blots and densitometry analyses of (B) pAMPK, (C) pAKT, (D) IRp972 and (E) IRp1146. mRNA expression of (E) adiponectin and (F) PRDM16 in adipose tissue of WT and KO mice; * $p < 0.05$ vs. WT.

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

We showed a significant increase ($p < 0.05$) in Rev-Erb α 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).

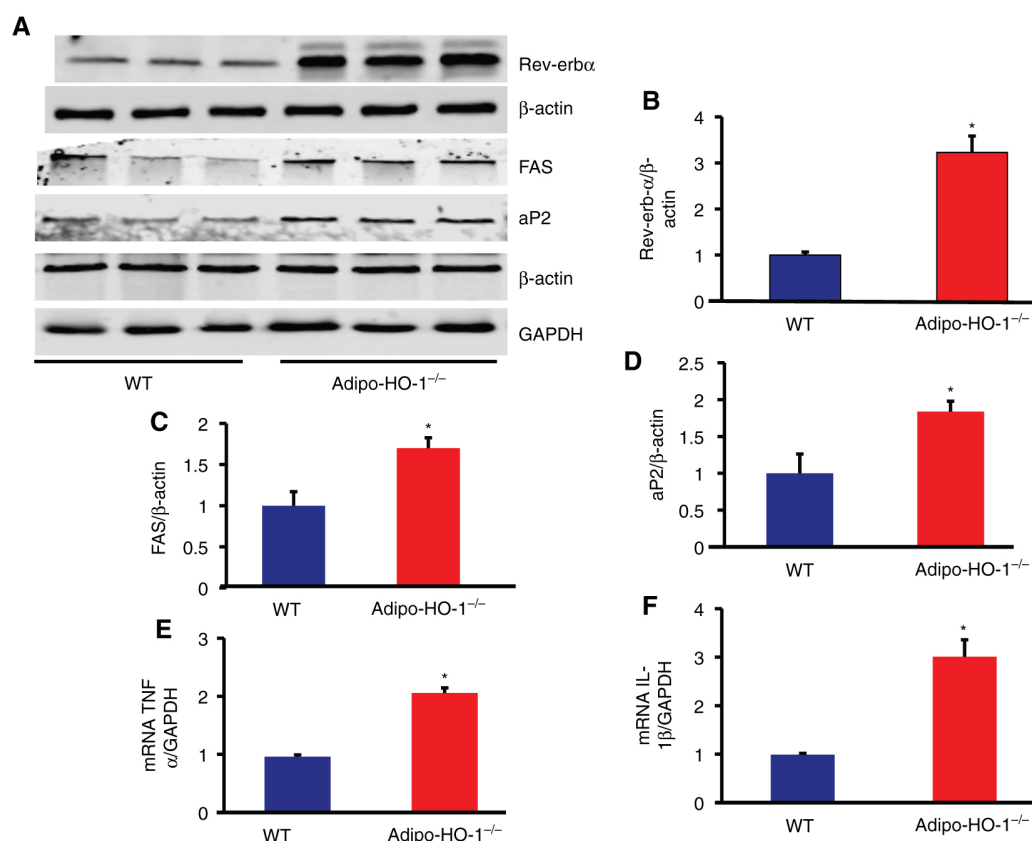


Figure 8: (A) Western blots and densitometry analyses of (B) Rev-Erbα, (C) FAS, (C) aP2 and mRNA expression of (E) TNFα and (F) IL-6 in adipose tissue of WT and KO mice; * $p < 0.05$ vs. WT.

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).

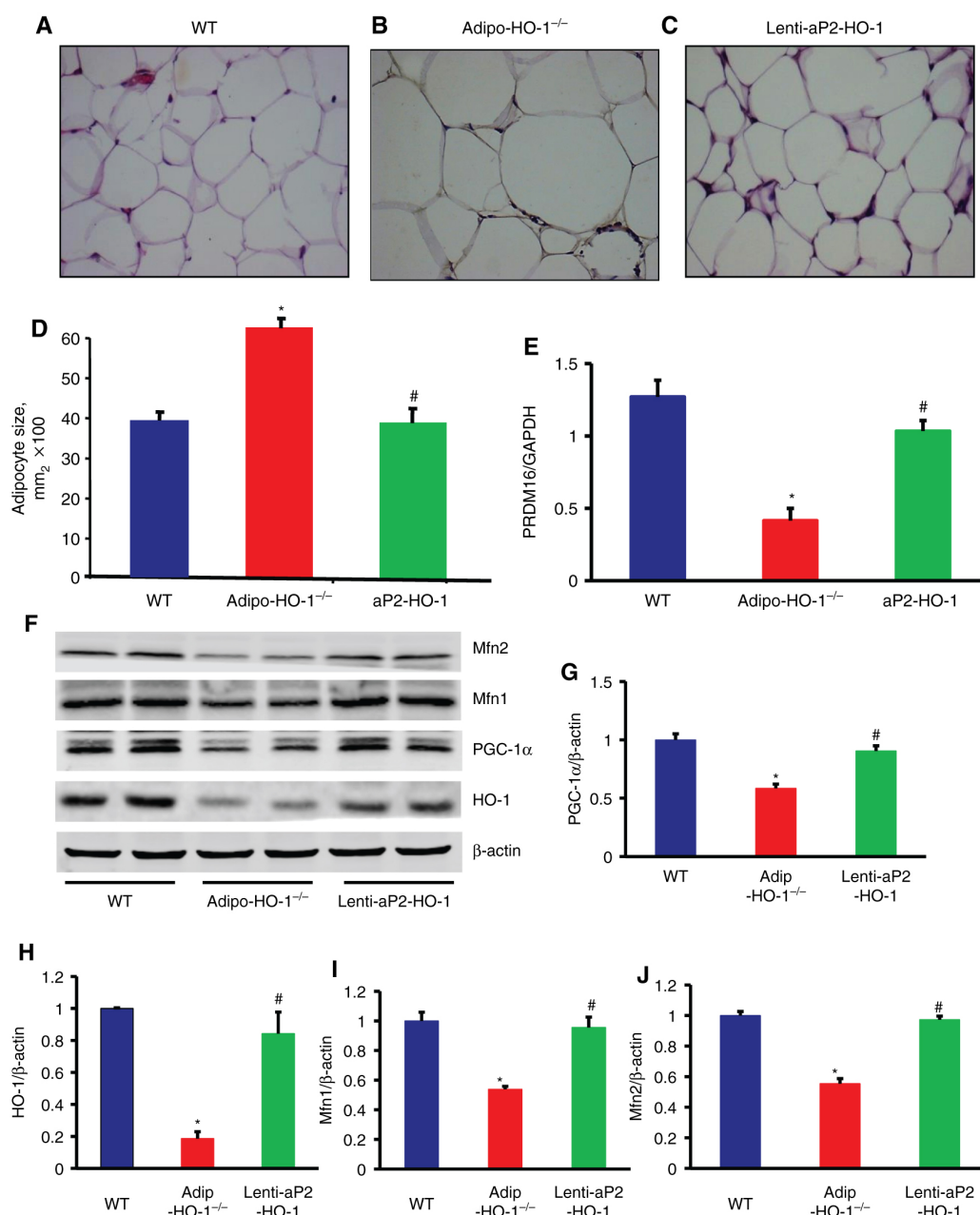


Figure 9: Hematoxylin-eosin depicts adipocyte size of WT (A), adipo-HO-1null (B) and (C) adipo-HO-1 over expressed (D) adipocyte measurements in adipose tissue of WT, KO and HO-1 overexpressed mice; $n = 10-15$. (E) mRNA expression of PRDM16, (F) Western blots and densitometry analyses of (G) PGC1 α , (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 O₂ 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-1 α levels and important genes that belong to beige and brown adipocytes [30], [45]. Reduction of PGC-1 α levels is associated with insulin resistance and hyperglycemia [46] and a decrease of mitochondrial oxidation phosphorylation and metabolic regulators [45], [47]. PGC-1 α 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-agonist-mediated HO-1 induction ameliorates progression of cardiomyopathy [58].

Spiegelman's group elegantly showed that PGC1 α 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 PGC1 α , the latter is essential to restore mitochondrial integrity and increase beige like cells. In contrast, induction of HO-1 increases heme turnover, increases PGC1 α , 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).

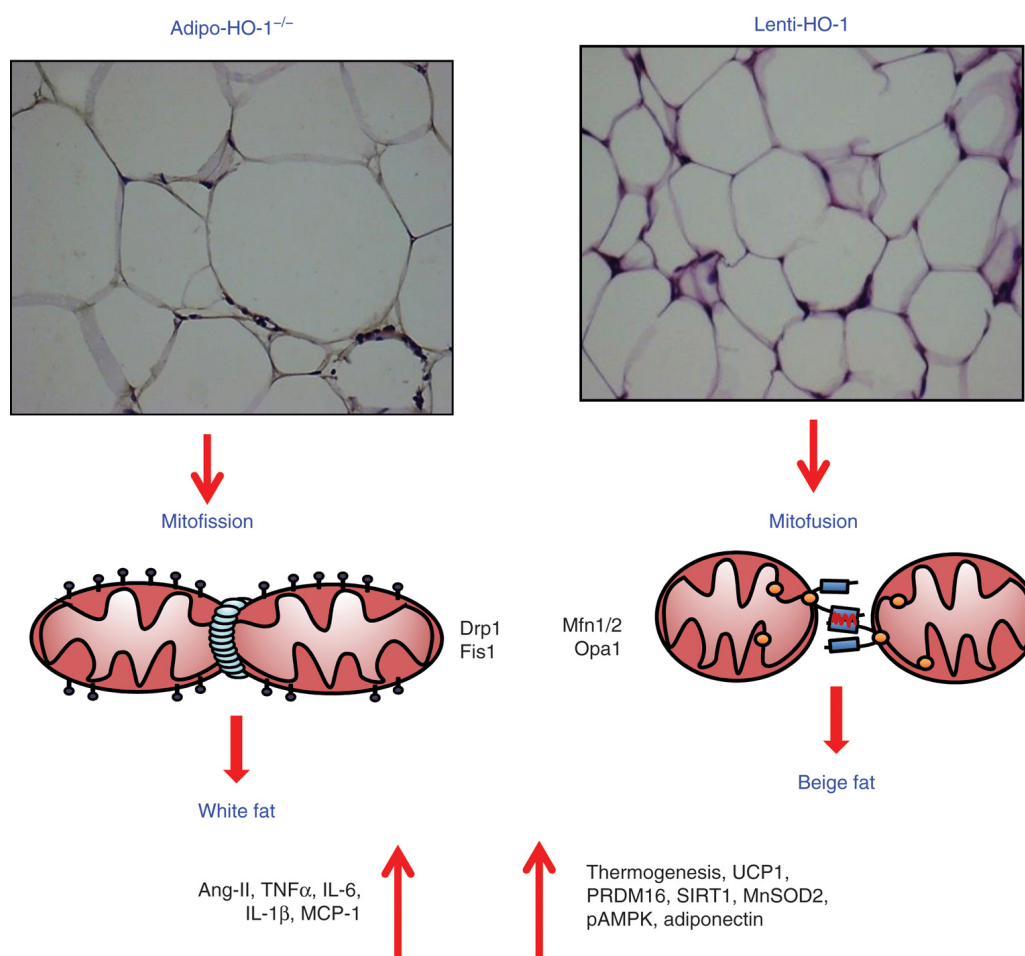


Figure 10: Schematic description showed that HO-1 gene ablation leads to an increase of adipogenic FAS, MEST and aP2 expression, in adipose tissue which is responsible for elevated adipocyte expansion, hypertrophy and releases inflammatory adipokines while decreasing PGC1.

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 PGC1 α , 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 PGC α , 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-1 α 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-1 α 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 PGC1 α 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.

Acknowledgements

We thank Mrs. Jennifer Brown for her outstanding assistance in preparing the manuscript.

Author Statement

Research funding: This work was supported by National Institutes of Health grant HL34300 (NGA) and The Renfield Foundation (AK). Conflict of interest: Authors state no conflict of interest. Informed consent: Informed

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.

References

- [1] Wang ZV, Scherer PE. Adiponectin, cardiovascular function, and hypertension. *Hypertension*. 2008;51:8–14.
- [2] Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. *Pharmacol Rev*. 2008;60:79–127.
- [3] Sodhi K, Srikanthan K, Coguet-Rubio P, Nichols A, Mallick A, Nawab A, et al. pNaKtide attenuates steatohepatitis and atherosclerosis by blocking Na/K-ATPase/ROS amplification in C57Bl6 and ApoE knockout mice fed a western diet. *Sci Rep*. 2017;7:193.
- [4] Quan S, Kaminski PM, Yang L, Morita T, Inaba M, Ikehara S, et al. Heme oxygenase-1 prevents superoxide anion-associated endothelial cell sloughing in diabetic rats. *Biochem Biophys Res Commun*. 2004;315:509–16.
- [5] Chang SH, Garcia J, Melendez JA, Kilberg MS, Agarwal A. Haem oxygenase 1 gene induction by glucose deprivation is mediated by reactive oxygen species via the mitochondrial electron-transport chain. *Biochem J*. 2003;371:877–85.
- [6] Chang SH, Barbosa-Tessmann I, Chen C, Kilberg MS, Agarwal A. Glucose deprivation induces heme oxygenase-1 gene expression by a pathway independent of the unfolded protein response. *J Biol Chem*. 2002;277:1933–40.
- [7] Kruger AL, Peterson SJ, Schwartzman ML, Fusco H, McClung JA, Weiss M, et al. Up-regulation of heme oxygenase provides vascular protection in an animal model of diabetes through its antioxidant and antiapoptotic effects. *J Pharmacol Exp Ther*. 2006;319:1144–52.
- [8] Kinobe R, Ji Y, Nakatsu K. Peroxynitrite-mediated inactivation of heme oxygenases. *BMC Pharmacol*. 2004;4:26.
- [9] Abraham NG, Rezzani R, Rodella L, Kruger A, Taller D, Li VC, et al. Overexpression of human heme oxygenase-1 attenuates endothelial cell sloughing in experimental diabetes. *Am J Physiol Heart Circ Physiol*. 2004;287:H2468–77.
- [10] Hinds TD, Sodhi K, Meadows C, Fedorova L, Puri N, Kim DH, et al. Increased HO-1 levels ameliorate fatty liver development through a reduction of heme and recruitment of FGF21. *Obesity (Silver Spring)*. 2014;22:705–12.
- [11] Nicolai A, Li M, Kim DH, Peterson SJ, Vanella L, Positano V, et al. Heme oxygenase-1 induction remodels adipose tissue and improves insulin sensitivity in obesity-induced diabetic rats. *Hypertension*. 2009;53:508–15.
- [12] Waldman M, Bellner L, Vanella L, Schragenheim J, Sodhi K, Singh SP, et al. Epoxyeicosatrienoic acids regulate adipocyte differentiation of mouse 3T3 cells, via PGC-1alpha activation, which is required for HO-1 expression and increased mitochondrial function. *Stem Cells Dev*. 2016;25:1084–94.
- [13] Abraham NG, Junge JM, Drummond GS. Translational significance of heme oxygenase in obesity and metabolic syndrome. *Trends in Pharmacological Sciences*. 2016;37(1):17–36.
- [14] Poss KD, Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci U S A*. 1997;94:10925–30.
- [15] Wenzel P, Rossmann H, Muller C, Kossmann S, Oelze M, Schulz A, et al. Heme oxygenase-1 suppresses a pro-inflammatory phenotype in monocytes and determines endothelial function and arterial hypertension in mice and humans. *Eur Heart J*. 2015;36:3437–46.
- [16] Cao J, Peterson SJ, Sodhi K, Vanella L, Barbagallo I, Rodella LF, et al. Heme oxygenase gene targeting to adipocytes attenuates adiposity and vascular dysfunction in mice fed a high-fat diet. *Hypertension*. 2012;60:467–75.
- [17] Vanella L, Kim DH, Sodhi K, Barbagallo I, Burgess AP, Falck JR, et al. Crosstalk between EET and HO-1 downregulates Bach1 and adipogenic marker expression in mesenchymal stem cell derived adipocytes. *Prostaglandins Other Lipid Mediat*. 2011;96:54–62.
- [18] Burgess A, Li M, Vanella L, Kim DH, Rezzani R, Rodella L, et al. Adipocyte heme oxygenase-1 induction attenuates metabolic syndrome in both male and female obese mice. *Hypertension*. 2010;56:1124–30.
- [19] Poss KD, Thomas MJ, Ebralidze AK, O'Dell TJ, Tonegawa S. Hippocampal long-term potentiation is normal in heme oxygenase-2 mutant mice. *Neuron*. 1995;15:867–73.
- [20] Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med*. 1996;2:87–90.
- [21] Morse D, Choi AM. Heme oxygenase-1: from bench to bedside. *Am J Respir Crit Care Med*. 2005;172:660–70.
- [22] Lundvig DM, Immenschuh S, Wagoner FA. Heme oxygenase, inflammation, and fibrosis: the good, the bad, and the ugly?. *Front Pharmacol*. 2012;3:81.
- [23] Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell*. 2012;150:366–76.
- [24] St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, et al. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. *J Biol Chem*. 2003;278:26597–603.
- [25] Yan M, Audet-Walsh E, Manteghi S, Dufour CR, Walker B, Baba M, et al. Chronic AMPK activation via loss of FLCN induces functional beige adipose tissue through PGC-1alpha/ERRalpha. *Genes Dev*. 2016;30:1034–46.
- [26] Ye L, Kleiner S, Wu J, Sah R, Gupta RK, Banks AS, et al. TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis. *Cell*. 2012;151:96–110.
- [27] Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev*. 2012;26:271–81.
- [28] Hattori K, Naguro I, Okabe K, Funatsu T, Furutani S, Takeda K, et al. ASK1 signalling regulates brown and beige adipocyte function. *Nat Commun*. 2016;7:11158.
- [29] Chung YW, Ahmad F, Tang Y, Hockman SC, Kee HJ, Berger K, et al. White to beige conversion in PDE3B KO adipose tissue through activation of AMPK signaling and mitochondrial function. *Sci Rep*. 2017;7:40445.
- [30] Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC-1alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;481:463–468.
- [31] Kajimura S, Seale P, Spiegelman BM. Transcriptional control of brown fat development. *Cell Metab*. 2010;11:257–62.

- [32] Leonardsson G, Steel JH, Christian M, Pocock V, Milligan S, Bell J, et al. Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc Natl Acad Sci U S A*. 2004;101:8437–42.
- [33] Seale P, Kajimura S, Spiegelman BM. Transcriptional control of brown adipocyte development and physiological function – of mice and men. *Genes Dev*. 2009;23:788–97.
- [34] Chang CR, Blackstone C. Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. *Ann N Y Acad Sci*. 2010;1201:34–39.
- [35] Lionetti L, Mollica MP, Donizzetti I, Gifuni G, Sica R, Pignalosa A, et al. High-lard and high-fish-oil diets differ in their effects on function and dynamic behaviour of rat hepatic mitochondria. *PLoS One*. 2014;9:e92753.
- [36] Alavi MV, Fuhrmann N. Dominant optic atrophy, OPA1, and mitochondrial quality control: understanding mitochondrial network dynamics. *Mol Neurodegener*. 2013;8:32.
- [37] Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. *Cell*. 2006;125:1241–52.
- [38] Di Noia MA, Van DS, Palmieri F, Yang LM, Quan S, Goodman AI, et al. Heme oxygenase-1 enhances renal mitochondrial transport carriers and cytochrome C oxidase activity in experimental diabetes. *J Biol Chem*. 2006;281:15687–93.
- [39] Ayer A, Zarjou A, Agarwal A, Stocker R. Heme Oxygenases in Cardiovascular Health and Disease. *Physiol Rev*. 2016;96:1449–508.
- [40] Bolisetty S, Traylor A, Zarjou A, Johnson MS, Benavides GA, Ricart K, et al. Mitochondria-targeted heme oxygenase-1 decreases oxidative stress in renal epithelial cells. *Am J Physiol Renal Physiol*. 2013;305:F255–64.
- [41] Hull TD, Boddu R, Guo L, Tisher CC, Traylor AM, Patel B, et al. Heme oxygenase-1 regulates mitochondrial quality control in the heart. *JCI Insight*. 2016;1:e85817.
- [42] Singh SP, Schragenheim J, Cao J, Falck JR, Abraham NG, Bellner L. PGC-1 alpha regulates HO-1 expression, mitochondrial dynamics and biogenesis: role of epoxyeicosatrienoic acid. *Prostaglandins Other Lipid Mediat*. 2016;125:8–18.
- [43] Singh SP, Bellner L, Vanella L, Cao J, Falck JR, Kappas A, et al. Downregulation of PGC-1alpha prevents the beneficial effect of EET-heme oxygenase-1 on mitochondrial integrity and associated metabolic function in obese mice. *J Nutr Metab*. 2016;2016:9039754.
- [44] Li M, Kim DH, Tsenovoy PL, Peterson SJ, Rezzani R, Rodella LF, et al. Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. *Diabetes*. 2008;57:1526–35.
- [45] Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev*. 2003;24:78–90.
- [46] Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab*. 2005;1:361–70.
- [47] Mootha VK, Handschin C, Arlow D, Xie X, Pierre JS, Sihag S, et al. Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. *Proc Natl Acad Sci U S A*. 2004;101:6570–5.
- [48] Cohen P, Levy JD, Zhang Y, Frontini A, Kolodin DP, Svensson KJ, et al. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell*. 2014;156:304–16.
- [49] Wu J, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: is beige the new brown?. *Genes Dev*. 2013;27:234–50.
- [50] Mitschke MM, Hoffmann LS, Gnad T, Scholz D, Kruthoff K, Mayer P, et al. Increased cGMP promotes healthy expansion and browning of white adipose tissue. *FASEB J*. 2013;27:1621–30.
- [51] Cohen P, Spiegelman BM. Brown and beige fat: molecular parts of a thermogenic machine. *Diabetes*. 2015;64:2346–51.
- [52] Peterson SJ, Kim DH, Li M, Positano V, Vanella L, Rodella LF, et al. The L-4F mimetic peptide prevents insulin resistance through increased levels of HO-1, pAMPK, and pAKT in obese mice. *J Lipid Res*. 2009;50:1293–304.
- [53] Suliman HB, Keenan JE, Piantadosi CA. Mitochondrial quality-control dysregulation in conditional HO-1^{-/-} mice. *JCI Insight*. 2017;2:e89676.
- [54] Balla J, Jacob HS, Balla G, Nath K, Eaton JW, Vercellotti GM. Endothelial-cell heme uptake from heme proteins: induction of sensitization and desensitization to oxidant damage. *Proc Natl Acad Sci U S A*. 1993;90:9285–9.
- [55] Handschin C, Lin J, Rhee J, Peyer AK, Chin S, Wu PH, et al. Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1alpha. *Cell*. 2005;122:505–15.
- [56] Ota U, Hara T, Nakagawa H, Tsuru E, Tsuda M, Kamiya A, et al. 5-aminolevulinic acid combined with ferrous ion reduces adiposity and improves glucose tolerance in diet-induced obese mice via enhancing mitochondrial function. *BMC Pharmacol Toxicol*. 2017;18:7.
- [57] Zhao M, Zhu P, Fujino M, Nishio Y, Chen J, Ito H, et al. 5-Aminolevulinic acid with sodium ferrous citrate induces autophagy and protects cardiomyocytes from hypoxia-induced cellular injury through MAPK-Nrf-2-HO-1 signaling cascade. *Biochem Biophys Res Commun*. 2016;479:663–9.
- [58] Cao Jian, et al. EET Intervention on Wnt1, NOV and HO-1 Signaling Prevents Obesity-Induced Cardiomyopathy in Obese Mice. *American Journal of Physiology - Heart and Circulatory Physiology*. 2017 6 2;ajpheart.00093.2017–ajpheart.00093.2017. DOI: 10.1152/ajpheart.00093.2017.