Adipocyte Heme Oxygenase-1 Induction Attenuates Metabolic Syndrome in Both Male and Female Obese Mice

Angela Burgess, Ming Li, Luca Vanella, Dong Hyun Kim, Rita Rezzani, Luigi Rodella, Komal Sodhi, Martina Canestraro, Pavel Martasek, Stephen J. Peterson, Attallah Kappas, Nader G. Abraham

Abstract—Increases in visceral fat are associated with increased inflammation, dyslipidemia, insulin resistance, glucose intolerance, and vascular dysfunction. We examined the effect of the potent heme oxygenase (HO)-1 inducer, cobalt protoporphyrin (CoPP), on regulation of adiposity and glucose levels in both female and male obese mice. Both lean and obese mice were administered CoPP intraperitoneally (3 mg/kg once per week) for 6 weeks. Serum levels of adiponectin, tumor necrosis factor α (TNFα), interleukin (IL)-1β and IL-6, and HO-1, PPARγ, pAKT, and pAMPK protein expression in adipocytes and vascular tissue were measured. While female obese mice continued to gain weight at a rate similar to controls, induction of HO-1 slowed the rate of weight gain in male obese mice. HO-1 induction led to lowered blood pressure levels in obese male and female mice similar to that of lean male and female mice. HO-1 induction also produced a significant decrease in the plasma levels of IL-6, TNFα, IL-1β, and fasting glucose of obese females compared to untreated female obese mice. HO-1 induction increased the number and decreased the size of adipocytes of obese animals. HO-1 induction increased adiponectin, pAKT, pAMPK, and PPARγ levels in adipocyte of obese animals. Induction of HO-1 in adipocytes was associated with an increase in adiponectin and a reduction in inflammatory cytokines. These findings offer the possibility of treating not only hypertension, but also other detrimental metabolic consequences of obesity including insulin resistance and dyslipidemia in obese populations by induction of HO-1 in adipocytes. (Hypertension. 2010;56:1124-1130.) • Online Data Supplement

Key Words: adipocyte pAMPK • female obesity • heme oxygenase inducers • inflammation

Moderate to severe obesity is associated with increased risk for cardiovascular complications and insulin resistance in humans1,2 and animals.3,4 Cardiovascular risk is specifically associated with increased intra-abdominal fat. Women in their reproductive years have a higher body mass index than males, which largely reflects increased overall subcutaneous adipose tissue or “gynoid” obesity; this is not associated with increased cardiovascular risk.5 However, because of increases in visceral fat with aging, after the age of 60 the fat distribution in females more closely resembles that in males.6 Increased androgen levels also often occur after the menopausal transition. These changes in visceral fat content and androgen levels correlate with both central obesity and insulin resistance and are an important determinant of cardiovascular risk.7

Heme oxygenase (HO) catalyzes the breakdown of heme, a potentially harmful pro-oxidant, into its products biliverdin and carbon monoxide, with a concomitant release of iron8. While HO-2 is expressed constitutively, HO-1 is inducible in response to oxidative stress, and its induction has been reported to normalize vascular and renal function.9-11 Furthermore, induction of HO-1 slows weight gain, decreases levels of tumor necrosis factor α (TNF-α) and interleukin (IL)-6, and increases serum levels of adiponectin in obese rats and obese diabetic mice.4,9,12 The association observed between HO-1 and adiponectin has led to the proposal of the existence of a cytoprotective HO-1/adiponectin axis.4,13

Previously, L’Abbate et al14 showed that induction of HO-1 is associated with a parallel increase in the serum levels of adiponectin, which has well-documented insulin-sensitizing, antiapoptotic, antioxidative, and anti-inflammatory properties. Adiponectin is an abundant protein secreted from adipocytes. Once secreted, it mediates its actions by binding to a set of receptors, such as adipor1 and adipor2, and also through induction of AMPK signaling pathways.15,16 In addition, increases in adiponectin play a protective role against TNF-mediated endothelial activation.17

In this study, we evaluated the effect of cobalt protoporphyrin (CoPP), a potent inducer of HO-1, on visceral and subcutaneous fat distribution in both female and male obese
mice. We show for the first time a resistance to weight reduction on administration of CoPP in female obese mice but a significant decrease in inflammatory cytokines. Despite continued obesity, CoPP normalized blood pressure levels, decreased circulating cytokines, and increased insulin sensitivity in obese females. CoPP treatment of obese mice increased the number and reduced the size of adipocytes. CoPP treatment of both male and female obese mice reversed the reduction in adiponectin levels seen in obesity. This study suggests that despite continued obesity, HO-1 induction in female obese mice serves a protective role against obesity-associated metabolic consequences via expansion of healthy smaller insulin-sensitive adipocytes.

Materials and Methods

Male and female obese mice (B6v-Lep ob/ob) were purchased from Harlan (Chicago, IL) at the age of 7 weeks. Lean mice (age-matched B6V, lean; Harlan) were used as the control. Sex-matched lean and obese mice were fed a normal laboratory animal diet and had free access to water. At 8 weeks of age after obese mice established diabetes, CoPP (3 mg/kg once per week) or stannous mesoporphyrin (SnMP; 3 mg/kg 3 times per week), a potent inhibitor of HO activity, was administered intraperitoneally for 6 weeks to 48 obese mice (24 males and 24 females) and 20 lean mice (10 males and 10 females). Measurements of glucose and insulin tolerance, body weight, and fasting blood glucose were made during the course of the study. Animal tissues and serum were then collected for additional studies.

For evaluation of adipocyte size analysis, digital images of adipose tissue sections were captured using a light microscope (Olympus) at ×20 magnification. For each group, 3 fields from each of 5 different hematoxylin-eosin-stained sections per animal were analyzed. Individual adipocyte areas (square micrometers) within each field were determined using image analysis software (Image Pro Plus; Imagini e Computer). For the quantitative analysis, adipocyte areas were calculated in arbitrary fields, measuring 50 adipocytes for each section. Other methodological details are provided in the online supplemental data (available at http://hyper.ahajournals.org). There was no difference in food intake in any of the treatment groups. The Animal Care and Use Committee of New York Medical College approved all experiments.

Results

Effect of Induction of HO-1 on Body Weight, Appearance, and Fat Content of Female and Male obese Mice

Previously, we have shown CoPP treatment results in the prevention of weight gain in several male models of obesity including obese and db/db mice and Zucker fat rats.4,12 We

Figure 1 (Continued). obese, and CoPP-treated obese mice. Bar, 50 μm/L. *P<0.05 compared to untreated obese mice. Bottom, Adipocyte size and number of adipocytes. VAT, *P<0.05 compared to lean or obese treated with CoPP. **P<0.05 vs obese. n=3 to 4 sections per group. B, Representative images of immunostained adipocytes from each experimental group. n=3 to 4 sections per group. Female immunohistochemistry of HO-1 and IOD determination of HO-1 expression in visceral aorta fat in lean, untreated obese, and CoPP- treated obese mice. *P<0.05 (similar results were seen in males; data not shown). C, Effect of CoPP on HO-1 protein levels in adipocyte isolated from pooled visceral fat of female lean and obese mice. Western blot and densitometry analysis of HO-1 protein in adipocyte isolated from fat tissues of lean and obese female mice treated with CoPP. Results are the mean±SE of the band density normalized to α-actin; n=4; *P<0.01 vs lean; #P<0.05 vs obese using 1-way ANOVA.
extended our studies to examine the effect of CoPP on weight gain in female obese mice. CoPP treatment prevented weight gain in male obese mice compared to age-matched male controls (Figure S1). The prevention of body weight gain was accompanied by a reduction in visceral fat in male obese mice. However, female obese mice administered CoPP did not lose weight but continued to gain weight at the same rate as untreated female obese mice (Figure S1). This was despite food intake being comparable between the 2 groups. CoPP administration decreased subcutaneous fat content in both obese males and females (P<0.05 and P<0.05, respectively). CoPP produced a decrease (P<0.05) in visceral fat in male but not in female obese mice compared to untreated obese mice (Figure S1D).

We examined adipocyte size by hematoxylin-eosin staining in lean, obese, and CoPP-treated obese female mice (Figure 1A, top). CoPP treatment resulted in a decrease in adipocyte size (P<0.05) compared with untreated obese animals (Figure 1A, top right). We then examined the number of adipocytes in lean, obese, and CoPP-treated obese female mice. The number of adipocytes (mean±SE) in lean, obese, and CoPP-treated obese animals was 40.83±3.50, 18.33±1.80, and 32.00±1.67, respectively, indicating that CoPP treatment of obese mice increased the number of adipocytes to levels similar to those in lean animals (Figure 1A, bottom right). Similar results were seen in male animals.

The induction of HO-1 was associated with a reduction in blood pressure. Systolic blood pressure in obese female mice was 142±6.5 mm Hg compared to obese CoPP treated (109±8.1 mm Hg, P<0.05). The value in obese female mice treated with CoPP is similar to the blood pressure seen in lean female mice (110±9.6 mm Hg). The systolic blood pressure in obese male mice was 144±4.5 mm Hg compared to obese CoPP treated (104±3.6 mm Hg, P<0.05).

We further examined whether CoPP affects HO-1 expression in adipocyte using immunohistochemistry and Western blot analysis. Immunostaining showed increased levels of HO-1 (green staining), located on the surface of adipocytes, after CoPP treatment (P<0.05), compared with female obese mice (Figure 1B). As seen in Figure 1C, HO-1 and HO-2 levels in adipocyte isolated from lean, untreated female obese mice or female obese mice treated with CoPP. Densitometry analysis showed that HO-1 was increased significantly in female obese mice treated with CoPP compared to nontreated female obese mice (P<0.05), which is in agreement with immunohistochemistry results. This pattern of HO expression in obesity occurs in other tissues, including aortas, kidneys, and hearts, of male obese mice.4,13
Effect of CoPP on HO-1 Expression and HO Activity in Female and Male obese Mice
HO-1 protein levels were increased by CoPP treatments in liver and renal tissues similar to that seen in adipocytes. Western blot analysis showed significant differences (P<0.05) in the ratio of HO-1 to actin in renal tissues of male and female obese and lean mice (Figure S2A). Obesity decreased HO-1 levels in both sexes compared to age-matched lean animals. In addition, HO-1 levels were significantly (P<0.05) lower in obese females compared to obese males (Figure S2A). This reflects a less active HO system in both male and female obese animals compared to age-matched lean controls. Next, we compared the effect of CoPP on male and female HO-1 gene expression in adipocytes. CoPP increased HO-1 expression in both male and female obese animals compared to untreated obese animals (Figure S2B) (P<0.001 and P<0.001, respectively). Similar results of HO-1 expression were seen in liver tissues (result not shown).

Effect of CoPP on Cytokine Levels in Female and Male obese Mice
CoPP administration resulted in a significant increase in the levels of plasma adiponectin in both female (P<0.001) and male (P<0.001) obese mice (Figure 2A). Untreated female obese animals exhibited a significant (P<0.05) increase in plasma IL-6 levels compared to age-matched male obese mice (Figure 2B). CoPP decreased plasma IL-6 levels in both female and male obese mice (P<0.05 and P<0.01, respectively) compared to untreated obese mice. Similar results were observed with plasma TNF-α and IL-1 levels (Figure 2C and 2D). These results indicate that although female obese mice exhibited elevated serum levels of inflammatory cytokines compared to male obese mice, CoPP acts with equal efficacy in both female and male obese animals in reducing inflammation while simultaneously increasing serum adiponectin levels (Figure 2).

Effect of CoPP on Blood Glucose and Low-Density Lipoprotein Levels in Female and Male obese Mice
Fasting glucose levels were determined after the development of insulin resistance. CoPP produced a decrease in glucose levels in both fasting female (P<0.05) and male (P<0.001) obese mice compared to untreated obese control animals (Figure 3A). CoPP reduced low-density lipoprotein (LDL) levels in both male (P<0.01) and female (P<0.05) obese mice compared to untreated obese controls (Figure 3B). Treatment with SnMP increased LDL levels. In separate experiments 2 weeks apart, glucose levels and insulin sensitivity were determined after development of insulin resistance (Figure 4A and B). Blood glucose levels in female obese mice were elevated (P<0.01) 30 minutes after glucose administration and remained elevated. In CoPP-treated female obese mice, blood glucose levels decreased significantly 60 to 120 minutes after glucose administration (P<0.01). Insulin administration to CoPP-treated female obese mice produced a decrease in glucose but not in the vehicle-treated female obese mice (P<0.01).

Effect of Obesity on Protein Expression Levels of pAKT, pAMPK, and PPARγ Levels in Female and Male obese Mice
Western blot analysis of adipocytes harvested from fat tissues, showed significant differences in basal protein expression levels of pAKT and pAMPK in untreated female obese mice compared to untreated male obese mice. pAMPK levels were higher in
Obese females compared to obese males (Figure 5A) \((P<0.05)\). This was also the case for pAKT protein levels, where increased levels of pAKT were seen in obese females compared to obese males (Figure 5B) \((P<0.05)\). CoPP treatment increased pAMPK and pAKT levels in both obese females and obese males. In addition, CoPP administration increased PPAR\(\gamma\) levels in both male \((P<0.001)\) and female \((P<0.05)\) obese mice (Figure 5C).

**Discussion**

In the current study, we show for the first time that induction of HO-1 regulates adiposity in both male and female animals via an increase in adipocyte HO-1 protein levels. A second novel finding is that induction of HO-1 was associated not only with a decrease in adipocyte cell size but with an increase in adipocyte cell number. Furthermore, induction of HO-1 affects visceral and subcutaneous fat distribution and metabolic function in male obese mice differently than in female obese mice. Despite continued obesity, upregulation of HO-1 induced major improvements in the metabolic profile of female obese mice exhibiting symptoms of type 2 diabetes including high plasma levels of proinflammatory cytokines, hyperglycemia, dyslipidemia, and low adiponectin levels. CoPP treatment resulted in increased serum adiponectin levels and decreased blood pressure. Adiponectin is exclusively secreted from adipose tissue, and its expression is higher in subcutaneous rather than in visceral adipose tissue. Increased adiponectin levels reduce adipocyte size and increase adipocyte number, resulting in smaller, more insulin-sensitive adipocytes. Adiponectin has recently attracted much attention because it has insulin-sensitizing properties that enhance fatty acid oxidation, liver insulin action, and glucose uptake and positively affect serum triglyceride levels. Levels of circulating adiponectin are inversely correlated with plasma levels of oxidized LDL in patients with type 2 diabetes and coronary artery disease, which suggests that low adiponectin levels are associated with an increased oxidative state in the arterial wall. Thus, increases in adiponectin mediated by upregulation of HO-1 may account for improved insulin sensitivity and reduced levels of LDL and inflammatory cytokines (TNF-\(\alpha\), IL-1\(\beta\), and IL-6 levels) in both male and female mice.

Females continued to gain weight despite the metabolic improvements. One plausible explanation for this anomaly is the direct effects of HO-1 on adiponectin mediating clonal expansion of preadipocytes. This supports the concept that expansion of adipogenesis leads to an increased number of adipocytes of smaller cell size; smaller adipocytes are considered to be healthy, insulin-sensitive adipocyte cells that are capable of producing adiponectin. This hypothesis is supported by the increase in the number of smaller adipocytes seen in CoPP-treated female obese animals without affecting weight gain compared to female obese animals. Similar results for the presence of HO-1–mediated smaller adipocytes were seen in males indicating that this effect is not sex specific.

Upregulation of HO-1 was also associated with increased levels of adipocyte pAKT and pAMPK and PPAR\(\gamma\) levels. Previous studies have indicated that insulin resistance and impaired PI3K/pAKT signaling can lead to the development of endothelial dysfunction. In the current study, increased

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**Figure 4.** Effect of HO-1 expression on glucose tolerance and insulin sensitivity. A, Intraperitoneal glucose tolerance (IPGTT). B, Intraperitoneal insulin sensitivity (IPITT) The results are means±SE. n=6 to 8 mice per group. \(*P<0.01\) vs female obese.
HO-1 expression was associated with increases in both AKT and AMPK phosphorylation; these actions may protect renal arterioles from insulin-mediated endothelial damage. By this mechanism, increased levels of HO-1 limit oxidative stress and facilitate activation of an adiponectin-pAMPK-pAKT pathway and increased insulin sensitivity. Induction of adiponectin and activation of the pAMPK-AKT pathway has been shown to provide vascular protection. A reduction in AMPK and AKT levels may also explain why inhibition of HO activity in CoPP-treated obese mice increases inflammatory cytokine levels while decreasing adiponectin. The action of CoPP in increasing pAKT, pAMPK, and PPARγ is associated with improved glucose tolerance and decreased insulin resistance. Insulin resistance is an independent factor for the development of both endothelial and vascular dysfunction. CoPP treated improved vascular function as manifest by increases in both insulin sensitivity and pAKT and pAMPK levels. Others have shown that increased phosphorylation of insulin receptors and vascular function may be a response to the increase in pAMPK and pAKT cross-talk. Further activation of pAMPK and pAKT increase glucose transport, fatty acid oxidation, and mitochondrial function. pAKT and AMPK act as fuel sensors in the regulation of energy balance, and the resultant the cross-talk of AMPK-AKT has been shown to regulate NO bioavailability and vascular function. Furthermore, activated AMPK alone has been suggested as a therapeutic target to ameliorate endothelial dysfunction.

The novel effect of CoPP on the HO-1-adiponectin-pAKT-pAMPK module (ie, an increase in HO-1) increases in adiponectin, and the subsequent increase in AKT-AMPK cross-talk and signaling pathway provides a beneficial mechanistic basis for CoPP-mediated vascular protection. Thus, HO-1 expression appears capable of reprogramming adipocytes, resulting in the expression of a new phenotype containing adipocytes of reduced cell size, increased number, increase in pAMPK-pAKT and restored insulin sensitivity. Although CoPP caused induction of HO-1 in various tissues, it is HO-1 induction in adipocytes that may be crucial for reversal of adipocyte dysfunction. HO-1 upregulation in adipocytes increases the release of adiponectin, with subsequent improvement in insulin sensitivity and a marked decrease in inflammatory cytokines. Therefore, targeting adipocytes with an HO-1 gene, we might be able to address obesity-mediated metabolic derangements and restore perivascular and vascular function.

Perspectives

We have demonstrated that HO-1 induction in adipocyte stem cells not only ameliorates obesity-associated metabolic consequences, including hypertension independent of body weight, but improves glucose tolerance in both male and female obese mice. The ability of HO-1 to cause an increase in adipocyte cell number and expansion of healthy adipocytes in obese mice and to reduce inflammatory cytokines appears to be primarily responsible for these effects. This appears to involve HO-1, adiponectin, and the PAKT-pAMPK signaling pathway acting in unison. These novel findings underscore the importance of targeting HO-1 to attenuate hypertension, insulin resistance, dyslipidemia, and subsequent cardiovascular risk within obese populations.
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Disclosures
None.

References
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Adipocyte Heme Oxygenase-1 Induction Attenuates Metabolic Syndrome In Both Male And Female Obese Mice

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Supplementary Material

Materials and Methods

Animal Care and CoPP Administration
Male and female obese mice (B6v-Lep obese/J) were purchased from Harlan (Chicago, IL) at the age of 7 weeks. Lean mice, (age-matched B6.V, lean, Harlan Chicago, IL) were used as control. Sex matched lean and ob mice were fed a normal laboratory animal diet and had free access to water. Body weight of obese and lean mice were 34 ± 5 g and 26 ± 3 g, respectively and glucose levels were 229 ± 21 and 154 ± 9 mg/dl, respectively, at the start of the experiments. At 8 weeks of age after obese mice established diabetes, CoPP (3mg/kg/once a week), or SnMP (3mg/kg/three times a week), was administered intraperitoneally for 6 weeks to 48 obese mice (24 males and 24 females) and 20 lean mice (10 males and 10 females). There were eight groups of animals: A) male lean, B) male obese, C) male obese- CoPP treated, D) male obese-CoPP+ treated, E) female lean, F) female obese, G) female obese-CoPP treated, and H) female obese-CoPP+SnMP treated. There was no difference in food intake in any of the treatment groups. The Animal Care and Use Committee of New York Medical College approved all experiments.

Preparation of Tissue for Histological Analysis of Adipocyte Cell Size and Adipocyte Cell Number
Subcutaneous and visceral aortic adipose fat tissues were isolated and fixed with 4% paraformaldehyde for 24-30 hours at room temperature and embedded in paraffin. 5-µm thick paraffin-embedded tissue sections were then deparaffinized and rehydrated in graduated alcohol in distilled water. Digital images of adipose tissue sections were capture using a light microscope (Olympus, Germany) at 20X magnification. For each group, five fields from each of five different ematoxylin-eosin stained section per animal were analyzed. The number of adipocytes within each field was determined using image analysis software as previously described 1, 2 (Image Pro Plus, Milan, Italy). Data are presented as means ± SD. Differences between experimental groups were evaluated with ANOVA with Bonferroni corrections. Statistical significance was set at P<0.05.

HO-1 Immunohistochemistry
Alternate sections of adipose tissues were deparaffinized, rehydrated and then incubated in 1% bovine serum albumin; subsequently sections were incubated with polyclonal rabbit antibody against HO-1 (diluted 1:500; Stressgene Bioreagents, Victoria, BC, Canada) for 1h at room temperature and overnight at 4°C. Then, sections were labeled using anti-mouse Alexa Fluar 546 and anti-rabbit Alexa Fluar 488 conjugated secondary antibodies(1:200, Invitrogen, UK). Finally, the samples were counter-stained with DAPI, mounted and observed with a confocal microscope (LSM 510 Zeiss, Germany) at a final magnification of 40X. The immunofluorescent control was perform by omitting the primary antibody and in presence of isotype matched IgGs.
**Blood Pressure Measurements**

Blood pressure was measured by the tail-cuff method before and every 7 days after CoPP administration.

**Determination of HO Activity**

HO activity was assayed as described previously. Using a technique in which bilirubin, the end product of heme degradation, was extracted from chloroform, and its concentration was determined spectrophotometrically (dual UV/VIS beam spectrophotometer lambda 25; PerkinElmer Life and Analytical Sciences, Wellesley, MA) using the difference in absorbance at a wavelength from $\lambda$460 to $\lambda$530 nm with an absorption coefficient of 40 mM$^{-1}$cm$^{-1}$. Under these conditions, HO activity was linear with protein concentration, time-dependent, and substrate-dependent.

**Cytokine, Glucose and LDL Measurements**

Adiponectin (high molecular weight, HMW), TNFα, IL-1β, and IL-6 were determined in mouse serum using an ELISA assay (Pierce Biotechnology, Woburn, MA). Glucose was measured using an automated analyzer (Lifescan Inc., Milpitas, CA). Serum LDL (low density lipoprotein) levels were measured using LDL/VLDL Quantification Kits (Biovision, Mountainview, CA). The assays were performed according to manufacturer’s guidelines.

**Glucose and Insulin Tolerance Tests**

After a 12 hour fast, mice were injected intraperitoneally with glucose (2.0g/kg body weight) and for insulin tolerance test, mice were injected with insulin (2.0 units/kg). Blood samples were taken at various time points (0-120 min), and fasting blood glucose levels were measured. Due to limitation in blood volumes for glucose determinations measurement of glucose levels (fasting) in Figures 3 and 4 were carried out on different groups of animals 2 weeks apart.

**Western Blot Analysis of HO-1, pAMPK, pAKT, and PPARγ expression**

At sacrifice, subcutaneous and visceral fat in the abdomen (the visible mesenteric fat, fat around the liver, the kidney, the spleen and the heart were dissected, pooled for each mouse and used to isolate adipocyte cells. Specimens were stored at -140°C until assayed. To isolate mouse adipocyte cells, adipose tissues were washed with phosphate-buffered saline (PBS) and digested at 37°C for 30 min with 0.075% type II collagenase. Frozen tissues were pulverized and placed in a homogenization buffer as previously described. Homogenates (20-50 μg of protein) were examined by protein immunoblot. HO-1, HO-2, AMPK, pAMK, AKT, pAKT, and PPAR gamma (Cell Signaling) levels were determined as previously described.

**Statistical analyses**

Statistical significance between experimental groups was determined by the Fisher method of analysis of multiple comparisons (p<0.05). For comparisons among treatment groups, the null hypothesis was tested by a two-factor ANOVA for multiple groups or unpaired $t$ test for two groups. Data are presented as mean ±SE.
Figure S1

A  Male obese  Male obese +CoPP  Female obese  Female obese +CoPP

B  

Body weight (g)  

Weeks  

Male obese  Male obese + CoPP  Female obese  Female obese + CoPP

*
Figure S1

C: Subcutaneous fat tissue weight / body weight

D: Visceral fat tissue weight / body weight
**Figure S1 A-D:** Effect of HO-1 induction on body weight, subcutaneous and visceral fat content in *obese* mice after 6 weeks of CoPP treatment (3mg/Kg/once a week) or vehicle solution.  
 **A)** *obese* male and female mice, respectively, were treated and weight determined (average of two independent experiments) n=6 for control and n=8 for treatment in each group, representative photograph for untreated male *obese*, male *obese* + CoPP, and untreated female *obese* and female *obese* + CoPP after 6 weeks of treatment.  
 **B)** Effect of CoPP treatment on body weight at the end of the treatment (6 weeks), p<0.05 versus male *obese*.  
 **C)** Effect of CoPP on subcutaneous fat. Results are by 2-way ANOVA. Levels of significance: *p<0.05 versus male *obese*; #p<0.05 versus female *obese*; **p<0.01 versus male *obese*; and ***p<0.01 versus male *obese* + CoPP.  
 **D)** Effect of CoPP on visceral fat. Level of significance: *p< 0.05 versus male *obese*; **p< 0.01 versus male *obese*. 

6
Figure S2

A

Male obese  Female obese
HO-1
β-actin

Male lean  Female lean

Relative Optical Density

HO-1/β-Actin

Male obese  Female obese

Male lean  Female lean
Figure S2

B

HO-1

β-actin

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Relative Optical Density

HO-1/β-actin

* Male Obese
* Female Obese
Figure S2: A, B) Expression of HO-1 in renal cells isolated from lean and obese female mice. Renal samples were subjected to Western blotting for the determination of HO-1 protein expression and densitometry analysis of HO-1/actin ratio. Results are expressed as means ± SE, n=4-6. Levels of significance: * p<0.05 versus untreated female obese mice, B) Effect of CoPP on HO-1 expression in renal cells of obese female mice, * p<0.001 versus obese; ** p<0.05 versus obese male; ***p<0.01 versus obese.
References


