

Research Article

Uric Acid-Induced Adipocyte Dysfunction Is Attenuated by HO-1 Upregulation: Potential Role of Antioxidant Therapy to Target Obesity

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Increased uric acid levels have been implicated in the pathogenesis of metabolic syndrome. To examine the mechanisms by which this occurs, we hypothesized that an increase in heme oxygenase 1, a potent antioxidant gene, will decrease uric acid levels and adipocyte dysfunction via suppression of ROS and xanthine oxidase (XO) levels. We examined the effect of uric acid on adipogenesis in human mesenchymal stem cells (MSCs) in the presence and absence of cobalt protoporphyrin (CoPP), an HO-1 inducer, and tin mesoporphyrin (SnMP), an HO activity inhibitor. Uric acid increased adipogenesis by increasing NADPH oxidase expression and elevation in the adipogenesis markers C/EBP α , PPAR γ , and Mest, while decreasing small lipid droplets and Wnt10b levels. We treated MSCs with fructose, a fuel source that increases uric acid levels. Our results showed that fructose increased XO expression as compared to the control and concomitant treatment with CoPP significantly decreased XO expression and uric acid levels. These beneficial effects of CoPP were reversed by SnMP, supporting a role for HO activity in mediating these effects. These findings demonstrate that increased levels of HO-1 appear crucial in modulating the phenotype of adipocytes exposed to uric acid and in downregulating XO and NADPH oxidase levels.

1. Introduction

Obesity is an epidemic that is becoming increasingly more prevalent, particularly in Western societies. Fructose, a dietary component, is often implicated in the development and exacerbation of obesity [1–6]. Much like glucose, fructose is a monosaccharide that is metabolized through the glycolytic pathway [7, 8]. However, unlike glucose, fructose is not primarily phosphorylated by hexokinase/glucokinase in the liver, but rather fructokinase, also known as ketohexokinase (KHK). KHK utilizes ATP to phosphorylate fructose to fructose-1-phosphate [2, 3]. This step is not regulated by insulin or fasting; furthermore, it bypasses the regulatory step in glycolysis catalyzed by phosphofructokinase. Therefore,

unregulated fructose metabolism produces uric acid as a byproduct through the induction of XO, thereby contributing to obesity and the potential for metabolic syndrome by increasing reactive oxygen species (ROS).

We hypothesized, as have others, that one mediator of fructose-induced symptoms of metabolic syndrome is uric acid [9–12]. Hyperuricemia is a well-known prelude to the development of many conditions, including gout and kidney disease [13]. Uric acid levels, highly predictive of body mass index (BMI) in humans, are elevated by many factors, including high purine diets and high fructose diets [10, 14]. While uric acid is a potent antioxidant, it can be highly damaging at elevated levels as seen in gout [15]. Ironically, its intracellular effect is to stimulate the production

of reactive oxygen species [16]. Intracellular uric acid has multiple origins, one of which is urate transport into cells through various transporters including URAT1, OAT, and SLC2A9 [13]. However, another origin of a more problematic nature is the KHK-dependent ATP depletion during fructose metabolism, which leads to a dramatic intracellular increase in purine degradation and uric acid. This pathway accelerates production of uric acid, as evidenced by diseases that inhibit purine recovery, such as Lesch-Nyhan syndrome. Uric acid induces oxidative stress via activation of NADPH oxidase [16]. NADPH oxidase increases production of O_2^- , resulting in increases in ROS, adipogenesis, insulin resistance, and inflammation [15, 17]. In addition, uric acid stimulates fructokinase and increases fructose breakdown [18]. This suggests that hyperuricemia is an indicator of adipocyte dysfunction in metabolic syndrome, and inducers of uric acid, especially fructose, may pose a serious threat to adipocyte function.

Imbalances in cellular redox status have been linked to adipose tissue dysfunction [4, 16]. It has been demonstrated that an NADPH oxidase inhibitor abolished the augmented ROS production accompanying adipogenesis in adipocytes [16, 19]. On the other hand, there is evidence that oxidative stress may promote adipogenesis via an upregulation of C/EBP α and PPAR γ in human bone marrow MSC [2, 20, 21]. The ROS produced by NADPH oxidase go on to activate the transcriptional factor Peg-1/Mest and induce adipocyte enlargement [22]. Induction of HO-1 decreases endogenous ROS and the protective effect of increased HO-1 expression is ascribed to several factors that include a reduction in cellular heme, the induction of the iron binding protein ferritin, and the increased formation of bilirubin, which efficiently scavenges toxic oxygen species [23]. Induction of HO-1 expression *in vivo* and *in vitro* is associated with an increase in preadipocytes, a reduction in the number of enlarged adipocytes, elevated adiponectin secretion, and an increase in the number of small adipocytes, which are regarded as “healthy” adipocytes [24]. HO-1 upregulates the transcriptional proteins β -catenin and Wnt10b that induce production of adiponectin, a hormone that keeps adipocytes small and healthy, and inhibits the activity of PPAR γ , a transcriptional factor that induces adipogenesis [25, 26].

The goal of this study was to determine whether fructose and uric acid-induced adipocyte dysfunction in MSC-derived adipocytes could be ameliorated by suppression of XO and NADPH oxidase by upregulation of HO-1. Our results showed that induction of HO-1 gene expression decreased lipid deposition and positive regulators of adipogenic markers and downregulated the expression of XO and NADPH oxidase. These findings demonstrate that increased levels of HO-1 appear crucial in modulating the phenotype of adipocytes exposed to uric acid and in suppressing markers of adipocyte hyperplasia and inflammation.

2. Material and Methods

2.1. Human Bone-Marrow-Derived MSC Culture. Frozen bone marrow mononuclear cells were purchased from All Cells (Emeryville, CA). After thawing, mononuclear cells (MSCs) were resuspended in α -minimal essential medium

(α -MEM, Invitrogen, Carlsbad, CA) that was supplemented with 20% FBS and 1% A/A. The cells were transferred into a 75-cm² flask, cultured in α -MEM with 20% FBS and 1% A/A, and maintained at 37°C in a 5% CO₂ incubator. The medium was changed after 24 hours and every 2 days thereafter. Once being 80% confluent, the MSCs were recovered by the addition of 0.25% trypsin/EDTA, split, and partitioned into additional 75 cm² flasks.

2.2. Human Bone-Marrow-Derived MSC Adipocyte Differentiation. MSCs (passages 2-3) were plated in 75 cm² flasks and 24-well plates at a density of $1-2 \times 10^4$ cells per cm² and were again cultured in their respective maintenance media until cells were 80% confluent. The maintenance medium was then replaced with adipogenic medium and the cells were cultured for an additional 14 days. The adipogenic medium consisted of complete culture medium supplemented with DMEM-high glucose (Invitrogen, Carlsbad, CA), 10% FBS, 1% A/A, 10 μ g/mL insulin, 1 μ M dexamethasone (Sigma-Aldrich, St. Louis, MO), and 0.1 mM Indomethacin (Sigma-Aldrich, St. Louis, MO). Human MSCs were cultured in this adipogenic differentiation medium along with fructose and uric acid in the presence and absence of the HO-1 inducer cobalt protoporphyrin (CoPP) (5 μ M), HO activity inhibitor tin-mesoporphyrin (SnMP) (5 μ M), xanthine oxidase inhibitor allopurinol (50 μ M) (Sigma-Aldrich, St. Louis, MO), and NADPH oxidase inhibitor apocynin (100 μ M) (Sigma-Aldrich, St. Louis, MO). The medium and treatments were renewed daily.

2.3. Fructose and Uric Acid Concentrations on Adipogenesis. To determine the optimal effect of fructose and uric acid on MSC-derived adipocyte differentiation, cells were treated with fructose at concentrations of 500 and 1000 μ M/L and uric acid at concentrations of 5 and 10 mg/dL, respectively. After 14 days, cells were stained with Oil Red O solution. Optimum concentrations for fructose and uric acid were found to be 500 μ M/L and 5 mg/dL, respectively. These concentrations were utilized during treatment of cells throughout experimentation.

2.4. Oil Red O Staining. 0.21% Oil Red O in 100% isopropanol (Sigma-Aldrich, St. Louis, MO) was used for staining. Briefly, adipocytes were fixed in 4% formaldehyde, stained with Oil Red O for 10 min, and rinsed with pH 7.2 PBS (1x) (Invitrogen, Carlsbad, CA). Pictures were captured using an Olympus IX81 Motorized Inverted Microscope. Oil Red O was eluted by adding 100% isopropanol for 10 min and OD was measured at 490 nm 24 hours later.

2.5. Measurement of Lipid Droplet Size. Cell size was measured using ImagePro Analyzer (Media Cybernetics, Inc., MD). The classification of the size of lipid droplets was based on size by area (pixels).

2.6. Measurement of MSC-Derived Adipocyte Signaling Molecules. Cells were maintained at -80°C until required for assay. Frozen cells were pulverized and placed in a homogenization buffer (10 mM phosphate buffer, 250 mM sucrose,

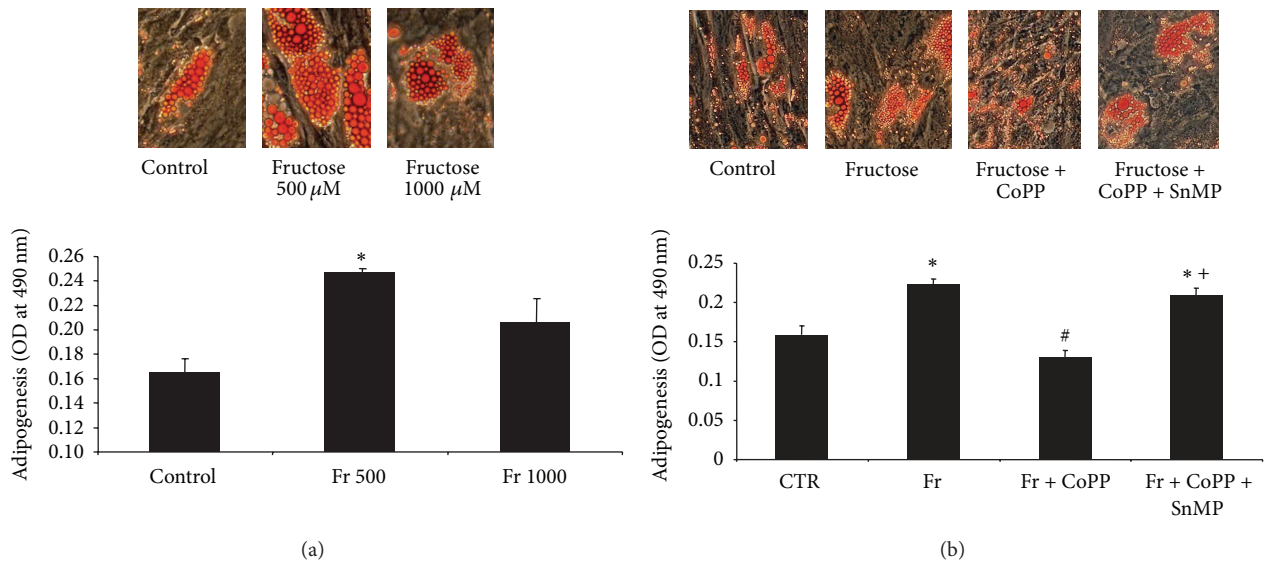


FIGURE 1: (a) Adipogenesis as shown by Oil Red O staining in MSCs treated with 500 μM and 1000 μM fructose. A representative section for each group is shown. (b) Adipogenesis as shown by Oil Red O staining in MSCs treated with 500 μM fructose with or without CoPP, in the presence or absence of SnMP, magnifications: 40x ($n = 5$). A representative section for each group is shown. Values represent means \pm SEM of five independent treatments. * $p < 0.05$ versus CTR; # $p < 0.05$ versus Fr; and + $p < 0.05$ versus Fr + CoPP.

1 mM EDTA, 0.1 mM PMSF, and 0.1% Tergitol, pH 7.5). Homogenates were centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was isolated and protein levels were assayed (Bradford method). The supernatant was used for measurement of XO, NADPH oxidase, and β -actin levels. β -actin was used to ensure adequate sample loading for all western blots.

2.7. Measurement of Superoxide Levels. MSC-derived adipocytes were cultured on 96-well plates until they achieved approximately 70% confluence. After treatment with or without uric acid (5 mg/dL) in the absence and presence of CoPP (5 μM) and SnMP (5 μM) for 2 days, the cells were incubated with 10 μM dihydroethidium (DHE) for 30 min at 37°C. Fluorescence intensity was measured using a Perkin-Elmer Luminescence Spectrometer at excitation/emission filters of 530/620 nm.

2.8. RNA Extraction and Real-Time PCR Experiments. Total RNA was extracted from MSC-derived adipocytes using RNeasy Protect Mini kit (QIAGEN, MD, USA) according to the manufacturer's instructions. Total RNA (1 μg) was transcribed into cDNA using GeneAmp kit (Applied Biosystems, Branchburg, NJ, USA) reverse transcription reagents. Total RNA was analyzed by a quantitative real-time polymerase chain reaction (qRT-PCR). Real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems) on a 7500 HT Fast Real-Time PCR System (Applied Biosystems). Specific primers used were HO-1, PPAR γ , FAS, C/EBP α , Wnt10b, Mest, and actin. Each reaction was performed in triplicate. The comparative threshold cycle (Ct) method was used to calculate the fold amplification as specified by the manufacturer. All experimental samples were normalized

using GAPDH as an internal control and normalization was performed in separate reactions.

2.9. Statistical Analyses. Statistical significance was determined using one-way analysis of variance followed by Tukey-Kramer post hoc test. $p < 0.05$ was considered to be significant. Data are expressed as means \pm standard error of the mean (SEM).

3. Results

3.1. Effect of Fructose on Adipogenesis in MSC-Derived Adipocytes. We measured adipogenesis in MSC-derived adipocytes after treatment with increasing concentrations of fructose. Fructose at a concentration of 500 $\mu\text{M/L}$ was determined to be the optimal concentration for stimulating adipogenesis in MSC-derived adipocytes as compared to the control (Figure 1(a); $p < 0.05$) as shown by us previously [27]. The same instrumental analysis was applied to MSC-derived adipocytes being treated with CoPP, an HO-1 agonist, which showed a reduction in adipogenesis to levels similar to the control group. When SnMP, an HO-1 activity inhibitor, was added to cells treated with both fructose and CoPP, adipogenesis increased to levels seen with only fructose treatment (Figure 1(b); $p < 0.05$).

3.2. Effect of HO-1 Induction on XO and Uric Acids Levels in MSC-Derived Adipocytes. Fructose metabolism invokes the consumption of ATP and thereby increases intracellular activity of XO and its product, uric acid. In concordance with our hypothesis, our results showed that fructose treatment of MSC-derived adipocytes significantly increased XO expression and uric acid to levels higher than the control

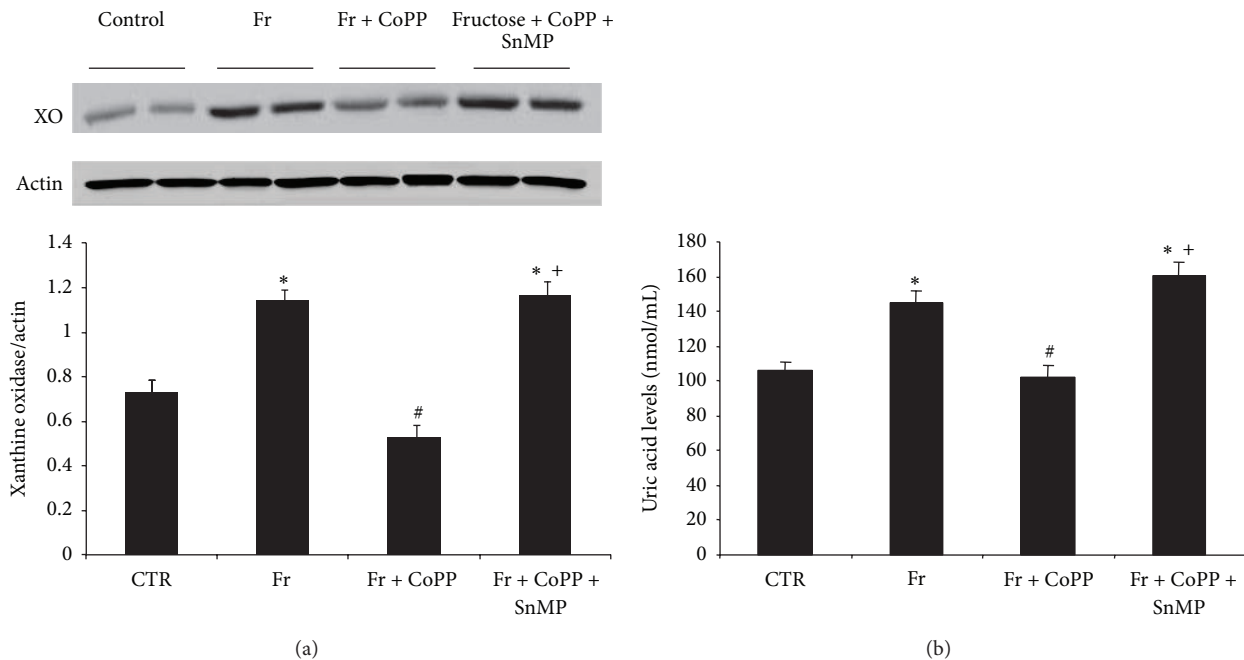


FIGURE 2: (a) XO expression by western blot analysis. Data are shown as mean band density normalized to β -actin. Results are means \pm SE, $n = 4$ /group. * $p < 0.05$ versus CTR; # $p < 0.05$ versus Fr; and + $p < 0.05$ versus Fr + CoPP. (b) Measurement of uric acid levels in pelleted cells at day 14 of adipogenesis in human MSCs treated with 500 μ M fructose with or without CoPP, in the presence or absence of SnMP. Values represent means \pm SEM of five independent treatments. * $p < 0.05$ versus CTR; # $p < 0.05$ versus Fr; and + $p < 0.05$ versus Fr + CoPP.

(Figures 2(a) and 2(b), resp.; $p < 0.05$). Concurrent treatment with fructose and CoPP effectively reversed both XO and uric acid levels to that of the control ($p < 0.05$). The addition of SnMP negated the beneficial effects of CoPP and increased XO and uric acid levels.

3.3. Effect of Uric Acid on Adipogenesis in MSC-Derived Adipocytes. We studied whether uric acid might be mediating fructose-induced adipogenesis. A concentration curve was obtained by treating cultured MSCs with uric acid concentrations of 5 mg/dL and 10 mg/dL. We demonstrated that cells treated with uric acid at a concentration of 5 mg/dL showed the maximum adipogenesis as compared to the control (Figure 3(a); $p < 0.05$). After observing an increase in adipogenesis in cells treated with uric acid, we then treated MSCs concurrently with uric acid and CoPP to confirm that HO-1 decreased uric acid-mediated adipogenesis. CoPP administration was shown to negate the adipogenic effect of uric acid and reduce adipogenesis to that of the control (Figure 3(b); $p < 0.05$). Our results further showed that SnMP, an HO activity inhibitor, reversed the beneficial effect of CoPP, resulting in increased adipogenesis (Figure 3(b); $p < 0.05$).

3.4. Effect of Uric Acid on Lipid Droplet Size and Mest and FAS Levels in MSC-Derived Adipocytes. The number of large, inflamed lipid droplets and small, healthy lipid droplets were measured in cells treated with uric acid (Figure 4(a)). Uric acid was shown to increase the number of large lipid droplets (Figure 4(b)) and decrease the number of small droplets as

compared to the control (Figure 4(c); $p < 0.05$). However, the concurrent administration of CoPP completely reversed this effect and decreased large droplets and increased small droplets when compared to control ($p < 0.05$). Concurrent treatment with SnMP reversed the beneficial effects of CoPP and the number of large lipid droplets was measured to be higher than that of the control (Figure 4(a); $p < 0.05$). Furthermore, our results showed that uric acid increased Mest and FAS expression levels compared to the control (Figures 4(d) and 4(e), resp.; $p < 0.05$). CoPP reversed the effects of uric acid and decreased Mest and FAS expression as compared to the cells treated with uric acid alone.

3.5. Effect of Uric Acid on HO-1 and Adipogenic Markers in MSC-Derived Adipocytes. Uric acid reduced expression of Wnt10b but had no effect on HO-1 expression. The effects of uric acid were reversed upon addition of CoPP, which increased HO-1 and Wnt10b expression to levels above the control (Figures 5(a) and 5(b), resp.; $p < 0.05$). When these MSCs were treated with the combination of uric acid, CoPP, and SnMP, the expression of Wnt10b was reduced to that of control. HO-1 expression remained unchanged as expected, given that SnMP modifies HO-1 activity and not its expression.

The expression of C/EBP α and PPAR γ , known mediators of adipocyte hypertrophy, were also measured by RT-PCR. Treatment of MSCs with uric acid increased expression of both C/EBP α and PPAR γ (Figures 5(c) and 5(d), resp.; $p < 0.05$), and the levels of these proadipogenic mediators were reduced with addition of CoPP. When SnMP was added to

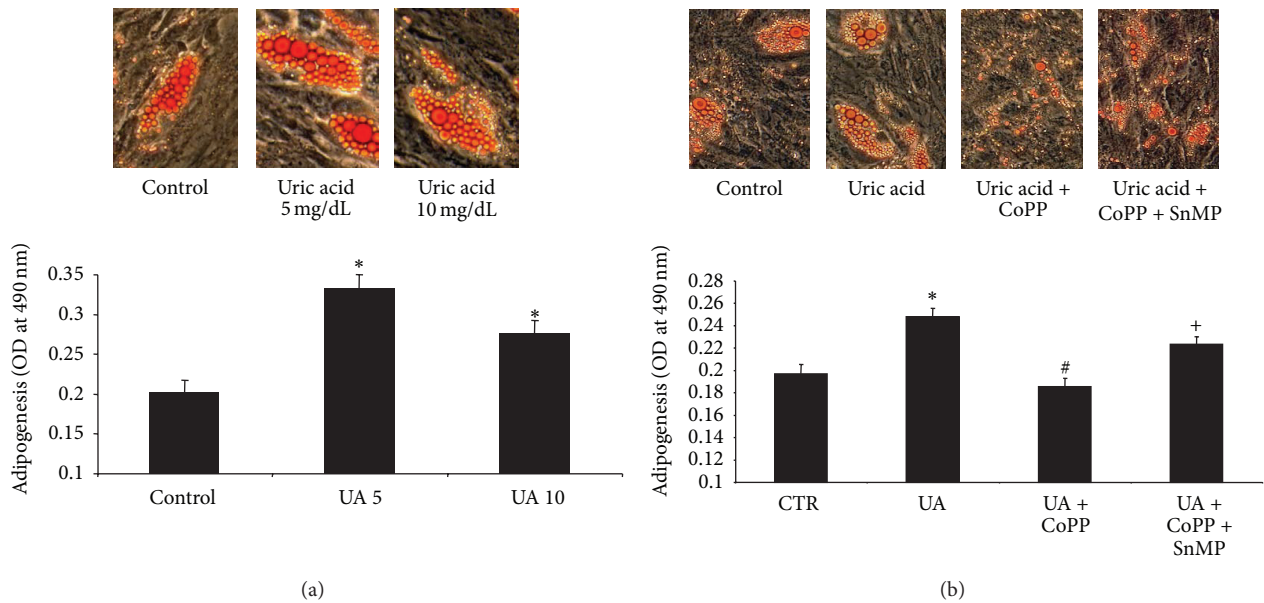


FIGURE 3: (a) Adipogenesis as shown by Oil Red O staining in MSCs treated with 5 mg/dL and 10 mg/dL uric acid. A representative section for each group is shown. (b) Adipogenesis as shown by Oil Red O staining in MSCs treated with 5 mg/dL uric acid with or without CoPP, in the presence or absence of SnMP, magnifications: 40x ($n = 5$). A representative section for each group is shown. Values represent means \pm SEM of five independent treatments. * $p < 0.05$ versus CTR; # $p < 0.05$ versus UA; and † $p < 0.05$ versus UA + CoPP.

MSCs treated with uric acid and CoPP, the protective factor of HO-1 was eliminated and the expression levels of PPAR γ and C/EBP α significantly increased as compared to the cells treated with uric acid and CoPP alone.

3.6. Effect of Uric Acid on Oxidative Stress in MSC-Derived Adipocytes. In order to better understand the mechanism by which uric acid was inducing adipogenesis and whether HO-1 induction plays an important role in attenuating uric acid-induced adipogenesis, NADPH oxidase expression and superoxide levels were measured. In MSC-derived adipocytes treated with uric acid, NADPH oxidase significantly increased compared to the control (Figure 6(a); $p < 0.05$). Similarly, superoxide levels were elevated in MSC-derived adipocytes treated with uric acid (Figure 6(b); $p < 0.05$). This oxidative stress was predicted to be an important cause of adipocyte dysfunction, so we treated cells with both uric acid and CoPP to see if HO-1 could decrease adipogenesis. Our results showed that CoPP significantly suppressed NADPH oxidase expression and superoxide levels as compared to cells treated with uric acid alone ($p < 0.05$). This positive outcome was effectively reversed by the addition of SnMP.

3.7. Inhibition of Fructose-Mediated Adipogenesis with Allopurinol and Uric Acid-Mediated Adipogenesis with Apocynin. First we sought to compare the effects on adipogenesis of CoPP and allopurinol, a known antagonist of XO. MSC-derived adipocytes were exposed to either fructose and allopurinol (100 mM) or fructose and CoPP, and adipogenesis was measured. Our results showed that allopurinol and CoPP attenuated adipogenesis at similar levels (Figure 7(a)). Then

we sought to compare the effects on adipogenesis of CoPP and apocynin (500 mM), a known inhibitor of NADPH oxidase. In a similar fashion, MSC-derived adipocytes were treated with the combination of uric acid and apocynin or uric acid and CoPP. Adipogenesis was inhibited in both treatment groups when compared to control ($p < 0.05$), and the magnitude of inhibition was relatively equal (Figure 7(b)).

4. Discussion

This study demonstrates the stimulatory effect of exogenous uric acid on adipogenesis in MSC-derived adipocytes, confirming their proadipogenic effects and the positive link between uric acid and the occurrence of obesity. This uric acid-mediated adipogenesis is attenuated by the induction of HO-1, suggesting its beneficial role in improving adipocyte function and decreasing XO and NADPH oxidase levels, thereby decreasing oxidative stress. The effects of uric acid were accompanied by increases in the proadipogenic factors Mest, FAS, C/EBP α , and PPAR γ , as well as decreases in the antiadipogenic factor Wnt10b; these findings are significantly reversed by treatment with CoPP. Thus, hyperuricemia stands as a potential contributing agent to obesity, and augmentation of HO-1 offers a promising avenue in attenuating adipocyte dysfunction.

Using MSCs as our study vehicle, we demonstrated that administration of either fructose or uric acid induced an increase in the amount of adipogenesis as compared to the control. These results verify that fructose and uric acid both have proadipogenic effects. Further our data demonstrated that MSC-derived adipocytes treated with uric acid produced a decreased expression of Wnt10b, which is essential in

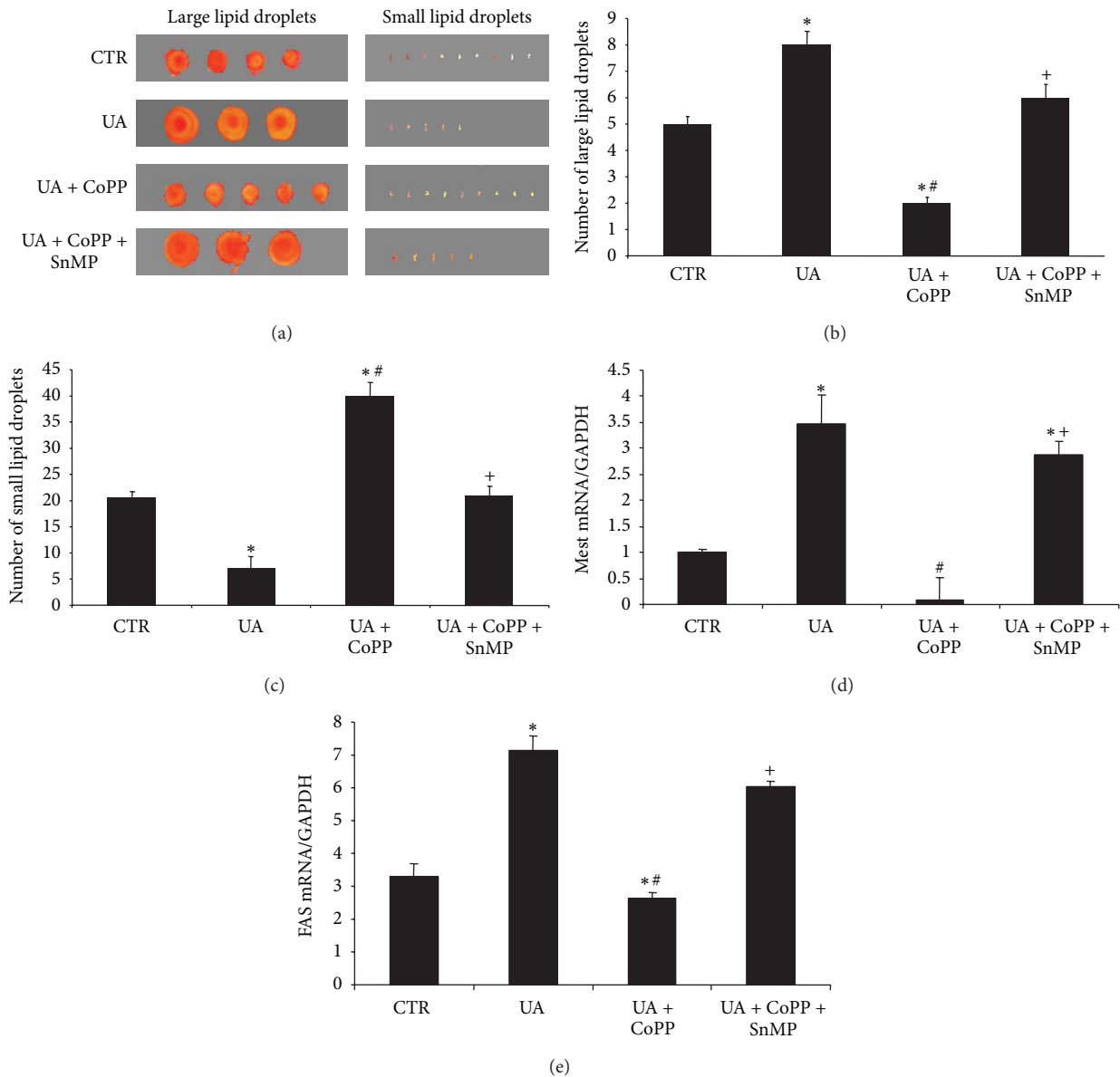


FIGURE 4: (a–c) Number of large and small lipid droplets from Oil Red O stained MSCs with 5 mg/dL uric acid with or without CoPP, in the presence or absence of SnMP; magnifications: 40x ($n = 3$). A representative section for each group is shown. Values represent means \pm SEM of five independent treatments. * $p < 0.05$ versus CTR; # $p < 0.05$ versus UA; and + $p < 0.05$ versus UA + CoPP. (d) Real-time PCR of Mest. (e) Real-time PCR of FAS expression in MSCs treated with uric acid with or without CoPP, in the presence or absence of SnMP. Values represent means \pm SEM of five independent treatments. * $p < 0.05$ versus CTR; # $p < 0.05$ versus UA; and + $p < 0.05$ versus UA + CoPP.

the activation of the canonical pathway and inhibition of adipogenesis [28, 29]. We also showed increased expression of C/EBP- α and PPAR γ , which has a crucial role in the transcriptional regulation of the adipocyte gene [21]. Moreover, our results showed that uric acid increased Mest expression, which is an important marker for enlargement of adipocytes during adipose tissue expansion [30]. Adipocyte enlargement is associated with an increase in the levels of inflammatory cytokines and oxidative stress [20, 24]. The morphologic changes induced by fructose and uric acid to the MSC-derived adipocytes support the link between excessive fructose intake and obesity.

Our results demonstrated that HO-1 induction attenuated adipogenesis induced by uric acid and fructose in MSC-derived adipocytes. One process by which fructose has been postulated to fuel such an increase in adipogenesis is the aforementioned unregulated ATP-dependent phosphorylation of fructose and subsequent buildup of uric acid via the purine degradation pathway [30]. Support for this theory stems from the result that uric acid, like fructose, is also proadipogenic. Since uric acid exerts its effects through ROS, HO-1, a potent antioxidant, was explored as a method of treatment. Induction of HO-1 decreases adipogenesis via suppression of transcription factors, including PPAR γ , aP2,

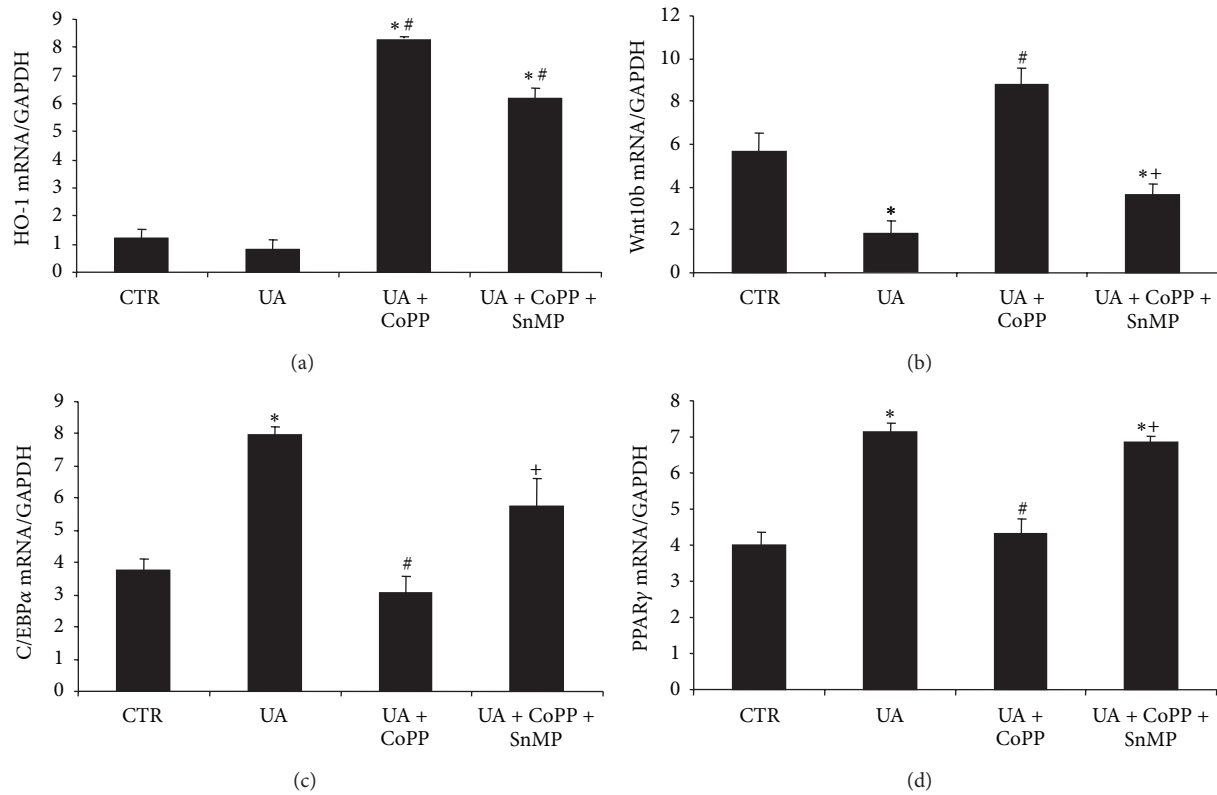


FIGURE 5: Real-time PCR of (a) HO-1; (b) Wnt10b; (c) C/EBP α ; and (d) PPAR γ expression in MSCs treated with uric acid with or without CoPP, in the presence or absence of SnMP. Values represent means \pm SEM of five independent treatments. * p < 0.05 versus CTR; # p < 0.05 versus UA; and + p < 0.05 versus UA + CoPP.

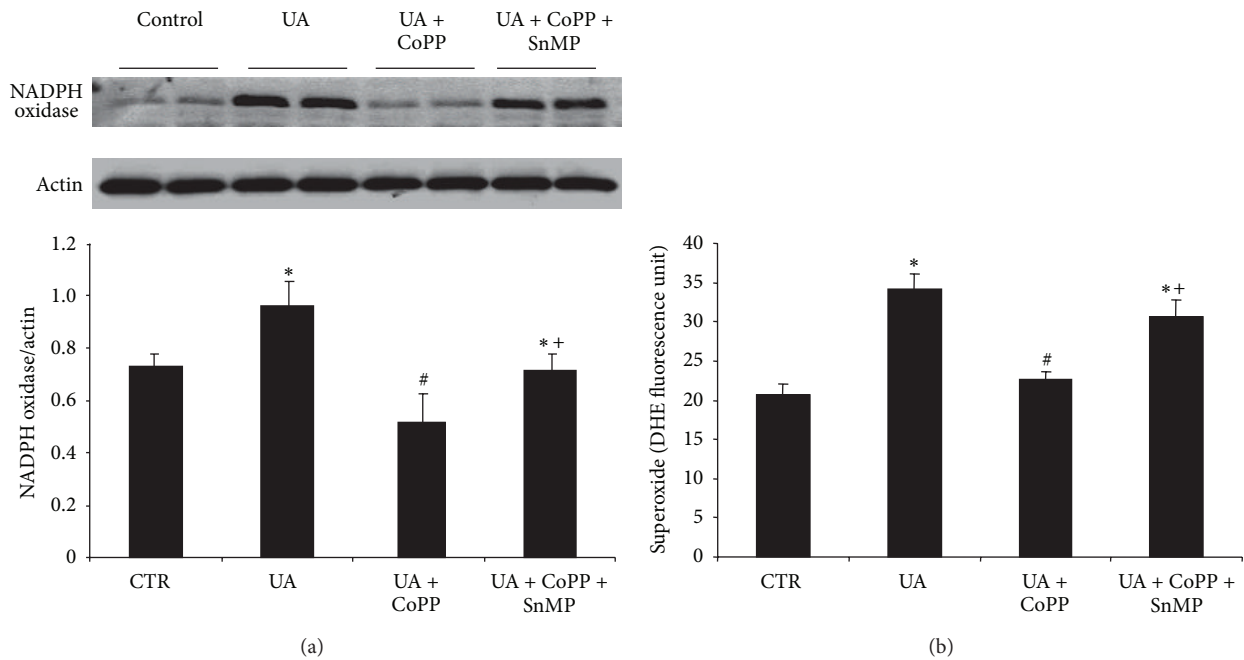


FIGURE 6: (a) NADPH oxidase expression by western blot analysis. Data are shown as mean band density normalized to β -actin. Results are means \pm SE, n = 4/group. * p < 0.05 versus CTR; # p < 0.05 versus UA; and + p < 0.05 versus UA + CoPP. (b) Measurement of superoxide levels in pelleted cells at day 14 of adipogenesis in human MSCs treated with 5 mg/dL uric acid with or without CoPP, in the presence or absence of SnMP. Values represent means \pm SEM of five independent treatments. * p < 0.05 versus CTR; # p < 0.05 versus UA; and + p < 0.05 versus UA + CoPP.

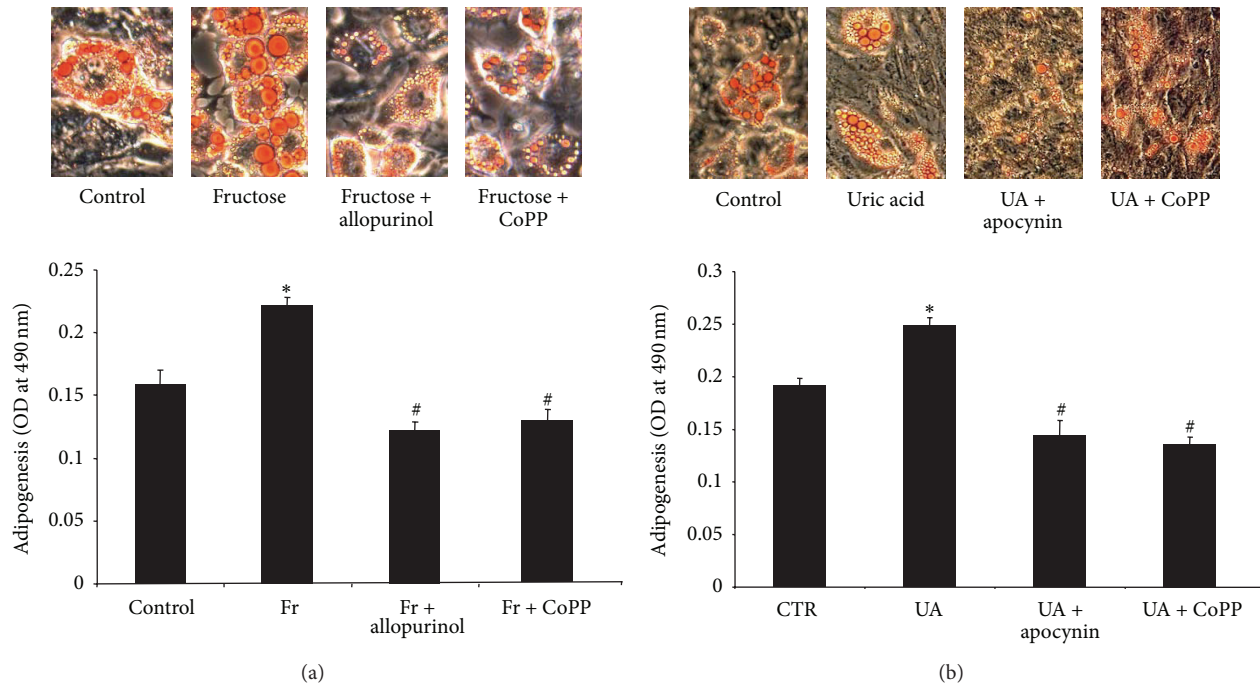


FIGURE 7: (a) Adipogenesis as shown by Oil Red O staining in MSCs treated with 500 μ M fructose with or without CoPP and with or without allopurinol, a known inhibitor of XO. A representative section for each group is shown; * $p < 0.05$ versus CTR; # $p < 0.05$ versus Fr. (b) Adipogenesis as shown by Oil Red O staining in MSCs treated with 5 mg/dL uric acid with or without CoPP and with or without apocynin, a known inhibitor of NADPH oxidase, magnifications: 40x ($n = 5$). A representative section for each group is shown. Values represent means \pm SEM of five independent treatments. * $p < 0.05$ versus CTR; # $p < 0.05$ versus UA.

and Mest proteins, while concomitantly increasing Wnt10b and adiponectin levels [25, 26, 28, 29, 31, 32]. We demonstrated in our study that upregulation of HO-1 counteracts the increase in adipogenesis resulting from treatment with fructose or uric acid in MSCs-derived adipocytes and also decreased adipocyte size, whereas inhibition of HO activity by SnMP increased adipocyte dysfunction. Our data further showed that the induction of HO-1 suppressed adipocyte differentiation, as evidenced by an increase in the canonical Wnt cascade and a decrease in Peg-1/Mest, FAS, and PPAR γ in uric acid-treated cells.

This study showed that induction of HO-1 via CoPP reduced expression of XO and NADPH oxidase and decreased oxidative stress-mediated adipogenesis in MSC-derived adipocytes. Uric acid produced in the presence of fructose via the upregulation of xanthine oxidase leads to an increase in oxidative stress. Our results showed for the first time that the MSC-derived adipocytes treated with fructose and CoPP downregulated XO expression. HO-1 induction also decreased uric acid levels, the final product of XO, further suggesting the beneficial role of HO-1 in attenuating XO-mediated oxidative stress and adipogenesis. Another potential mechanism by which HO-1 upregulation decreases adipogenesis in MSC-derived adipocytes may be related to suppression of NADPH oxidase, which contributes to oxidative stress. Bilirubin, a product of HO-1 catabolism, has been shown to scavenge ROS and play a role in the inhibition of NADPH oxidase [27]. Our results showed

that uric acid increased expression of NADPH oxidase in MSC-derived adipocytes, and concurrent administration of CoPP decreased its expression, suggesting that upregulation of HO-1 attenuates NADPH oxidase-mediated adipogenesis. NADPH oxidase also had the effect of upregulating the transcriptional factor Peg-1/Mest through the generation of the superoxide ion [33]. In concordance with these reports, our results showed that uric acid treatment in MSCs increased expression of Peg-1/Mest, and upregulation of HO-1 negated this increase in Peg-1/Mest expression. To further confirm our hypothesis that HO-1 upregulation plays an important role in attenuating XO- and NADPH oxidase-mediated adipogenesis, the efficacy of HO-1 was established by comparing its effect on adipogenesis to known antagonists of XO and NADPH oxidase, allopurinol and apocynin, respectively. Our results demonstrated that HO-1 induction displayed similar efficacy to that of both allopurinol and apocynin in terms of suppressing adipogenesis. These observations indicate that induction of HO-1 plays an important role in the prevention of adipocyte hypertrophy and the promotion of smaller healthier adipocytes in MSCs, in large part, through reduced expression of XO and NADPH oxidase. Our data underscores the importance of oxidative stress-induced adipocyte dysfunction as a result of uncontrolled fructose metabolism and offers new insights into potential therapies by which targeting fructose metabolism and/or downstream signaling of uric acid can curtail adipogenesis.

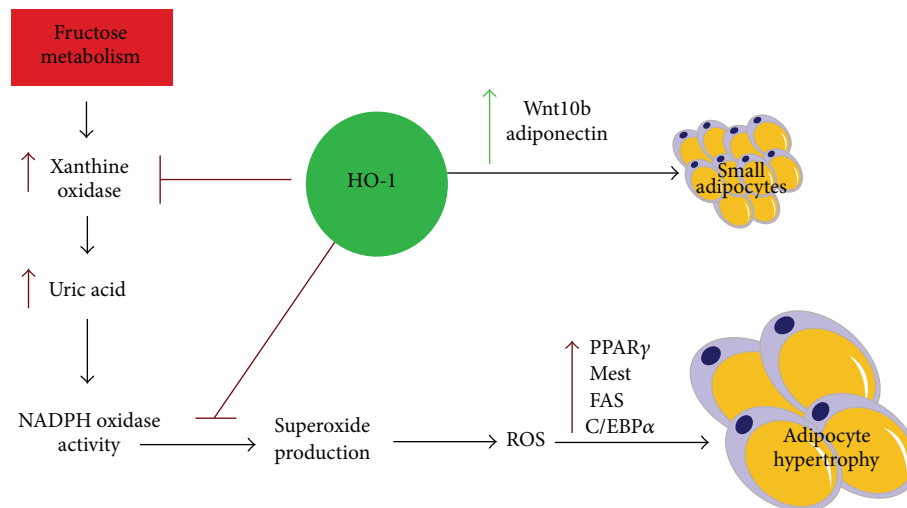


FIGURE 8: Proposed mechanism demonstrating the beneficial role of heme oxygenase 1 (HO-1) in the attenuation of adipocyte dysfunction in mesenchymal stem cell- (MSC-) derived adipocytes. Excessive fructose consumption is accompanied by an increase in xanthine oxidase and uric acid and a concurrent increase in cellular ROS levels. HO-1 suppresses adipocyte differentiation by increasing expression of key regulators including Wnt10b and adiponectin and decreasing expression of adipogenic transcription factors CCAAT/enhancer binding protein α (C/EBP α), peroxisome proliferator-activated receptor γ (PPAR γ), Mest, and FAS. These effects led to decrease in lipid accumulation and increase of preadipocytes and healthy adipocytes that improve adipocyte function.

5. Conclusion

In conclusion (Figure 8), we demonstrate that fructose increases adipogenesis in MSC-derived adipocytes. This effect is mediated through uric acid resulting from uncontrolled fructose metabolism, leading to upregulation of XO and NADPH oxidase. Given that obesity is correlated with increased blood levels of uric acid, this provides a potentially highly effective pharmacological intervention for overweight and obese individuals. It also highlights the crucial role of antioxidant therapy represented by HO-1 induction in targeting metabolic syndrome and obesity resulting from excessive fructose intake. Future research should seek to explore how treatment with HO-1 agonists may alter body mass index in patients suffering from obesity comorbid with increased plasma concentrations of uric acid. Investigation into this topic may prove to be extremely helpful in improving the health of Western society.

Abbreviations

XO: Xanthine oxidase
 C/EBP α : CCAAT/enhancer binding protein alpha
 CoPP: Cobalt protoporphyrin IX dichloride
 FAS: Fatty acid synthase
 HO: Heme oxygenase
 HO-1: Heme oxygenase isozyme 1, inducible form
 HO-2: Heme oxygenase isozyme 2, constitutive form
 MSC: Mesenchymal stem cell
 NADPH: Nicotinamide adenine dinucleotide phosphate (reduced form)

Peg-1/Mest: Paternally expressed gene 1/mesoderm-specific transcript
 PPAR γ : Peroxisome proliferator activated receptor gamma
 ROS: Reactive oxygen species
 SnMP: Tin mesoporphyrin IX dichloride
 Wnt10b: Wingless-type MMTV integration site family, member 10b.

Disclaimer

The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Jordan Hilgefert and George Banks contributed to work equally.

Acknowledgments

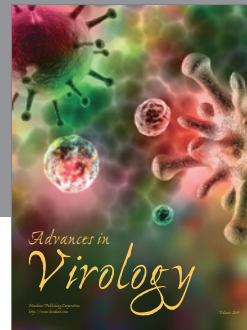
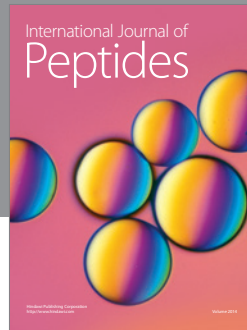
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