Hyperuricemia as a Mediator of the Proinflammatory Endocrine Imbalance in the Adipose Tissue in a Murine Model of the Metabolic Syndrome

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OBJECTIVE—Hyperuricemia is strongly associated with obesity and metabolic syndrome and can predict visceral obesity and insulin resistance. Previously, we showed that soluble uric acid directly stimulated the redox-dependent proinflammatory signaling in adipocytes. In this study we demonstrate the role of hyperuricemia in the production of key adipokines.

RESEARCH DESIGN AND METHODS—We used mouse 3T3-L1 adipocytes, human primary adipocytes, and a mouse model of metabolic syndrome and hyperuricemia.

RESULTS—Uric acid induced in vitro an increase in the production (mRNA and secreted protein) of monocyte chemotactic protein-1 (MCP-1), an adipokine playing an essential role in inducing the proinflammatory state in adipocytes in obesity. In addition, uric acid caused a decrease in the production of adiponectin, an adipocyte-specific insulin sensitizer and anti-inflammatory agent. Uric acid–induced increase in MCP-1 production was blocked by scavenging superoxide or by inhibiting NADPH oxidase and by stimulating peroxisome-proliferator–activated receptor-γ with rosiglitazone. Downregulation of the adiponectin production was prevented by rosiglitazone but not by antioxidants. In obese mice with metabolic syndrome, we observed hyperuricemia. Lowering uric acid in these mice by inhibiting xanthine oxidoreductase with allopurinol could improve the proinflammatory endocrine imbalance in the adipose tissue by reducing production of MCP-1 and increasing production of adiponectin. In addition, lowering uric acid in obese mice decreased macrophage infiltration in the adipose tissue and reduced insulin resistance.

CONCLUSIONS—Hyperuricemia might be partially responsible for the proinflammatory endocrine imbalance in the adipose tissue, which is an underlying mechanism of the low-grade inflammation and insulin resistance in subjects with the metabolic syndrome. ***Diabetes*** **60:1258–1269, 2011**

Elevated blood levels of uric acid, the final product of the purine degradation in humans, is strongly associated with cardiovascular and kidney disease, hypertension, and overall risk of mortality (1,2). It is also commonly present in metabolic syndrome (3). Serum levels of uric acid are positively correlated with individual components of the metabolic syndrome, in particular visceral obesity (4,5), and this correlation is stronger when other components are also present (5). In 1993 Reaven and colleagues (6) suggested that hyperuricemia is commonly part of the cluster of metabolic and hemodynamic abnormalities along with abdominal obesity, glucose intolerance, insulin resistance, dyslipidemia, and hypertension (7). Most authorities have viewed the presence of hyperuricemia in metabolic syndrome as a secondary response to obesity and hyperinsulinemia and have attributed this to the effects of insulin on proximal tubular urate transport (8), the effect of elevated leptin (9), or altered purine metabolism (10).

Recent studies, however, have suggested a potential contributory role of uric acid to metabolic syndrome. For example, an elevated serum uric acid has been reported to be an independent predictor of obesity (11) and hyperinsulinemia (12,13), and thus if it precedes the development of hyperinsulinemia, it is difficult to attribute it solely to the effects of elevated insulin levels. Lowering uric acid also ameliorates the elevation in blood pressure, serum triglycerides, and insulin resistance in the fructose-induced metabolic syndrome in rats (3,14). We showed recently that adipocyte differentiation is associated with an increase in uptake of uric acid by cells and, in differentiated adipocytes, uric acid induced an activation of NADPH oxidase (NOX) followed by the activation of redox-dependent proinflammatory signaling via protein kinase p38 (15).

Adipose tissue is important not only as a site for fat sequestration and storage, but also as a major endocrine gland that secretes hormones and cytokines referred to as adipokines (16,17). Adipocytes from lean and healthy subjects express high levels of adiponectin, which is secreted into the circulation, reaching serum concentrations of up to 10 μg/mL (18,19). Adiponectin stimulates fat oxidation, acts as an insulin sensitizer in many cell types (19,20), and has antiatherogenic properties (21). In obese subjects, adiponectin levels are decreased, and its beneficial effects are diminished (20,22,23). Obesity is also associated with an inflammatory response in the adipose tissue (24,25) with an increased local expression of monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and other proinflammatory molecules (23,25,26). MCP-1 has a key role in the macrophage infiltration in the adipose tissue in obesity and development of insulin resistance (27,28). The obesity-induced imbalance in the production of adipokines contributes to insulin resistance of the liver and muscles, impairs vascular homeostasis, and induces low-grade systemic inflammation that is critical in the development of

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type 2 diabetes and cardiovascular disease (18,19,29,30). Uric acid has also been reported to induce an increase in the expression of MCP-1 in some cell types (31,32) while clinical studies revealed a negative correlation between the levels of uric acid and adiponectin in the serum (33–35). In addition, uric acid is recognized by immune cells as a signal of cell death that alerts the immune system (36,37). Recently Cheung et al. (38) have reported that the xanthine dehydrogenase form of xanthine oxidoreductase (XOR), the enzyme that produces uric acid from xanthine, may also have a role in adipocyte differentiation.

Given these observations, we investigated whether uric acid might modulate the production of adiponectin and MCP-1 in the adipocyte and if this was dependent on local oxidative stress or peroxisome-proliferator–activated receptor-γ (PPAR-γ). We also examined whether hyperuricemia can be a factor contributing to the proinflammatory imbalance in the adipose tissue via effects on the MCP-1 and adiponectin production in a mouse model of the metabolic syndrome.

RESEARCH DESIGN AND METHODS

Cell culture and treatments. Mouse preadipocyte cells 3T3-L1 obtained from ATCC (Manassas, VA) were maintained in high glucose Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% FBS and antibiotics. For adipocyte differentiation, confluent cells were treated with 10 μg/mL insulin, 0.25 μmol/L dexamethasone, and 0.5 mmol/L isobutyl-1-methylxanhine for 2 days followed by 3- to 5-day treatment with insulin alone. Differentiation of adipocytes was confirmed by lipid staining with Red Oil and mRNA expression of PPAR-γ and adiponectin as described earlier (15). Human primary subcutaneous adipocytes were obtained from ZenBio (Research Triangle Park, NC) and cultured following the instruction of the manufacturer in subcutaneous adipocyte medium AM-1 (ZenBio). Uric acid solution for cell treatments (Ultrapure, 1–15 mg/dL; Sigma) was prepared in prewarmed cell culture medium as previously described (15). If treatments with uric acid were continued for more than 3 days, medium was replaced with freshly prepared identical medium. By the end of the 3-day treatment, there was no difference in pH between cell culture medium containing uric acid and control medium. In some experiments, conditioned medium was collected and stored at −80°C for measuring the levels of adipokines.

Animal model. The Animal Care and Use Committee of the University of Florida approved protocols for all animal experiments. To test the effect of lowering uric acid, we used the Pound mouse, a recently established prediabetes/metabolic syndrome model (Charles River, Wilmington, MA). These mice have a mutation in the leptin receptor (a deletion of the exon 2), and they develop obesity, insulin resistance, dyslipidemia, and fatty liver disease but do not progress to type 2 diabetes (39). In preliminary experiments we showed that these mice develop hyperuricemia (RESULTS). Obese (C57BL/6NCrl-Lepr$^{db\text{-}lb}$/Crl) and lean (C57BL/6NCrl) 6-week-old mice were maintained on a standard Purina 5001 Diet (PMI, Richmond, IN) under a 12-h light/12-h dark cycle with access to food and water ad libitum. To assess the effects of lowering uric acid levels, obese and lean animals (8–10 animals per group) were either administered for 8 weeks with allopurinol, an inhibitor of XOR (125 mg/L in the drinking water; Sigma, St. Louis, MO) or remained untreated. Every 3 weeks, samples of blood were obtained through facial vein to monitor the level of uric acid. Insulin tolerance testing was performed 1 week prior to the end of the experiment. Animals were fasted for 4 h and then injected intraperitoneally with insulin (Humulin R; Eli Lilly, Indianapolis, IN) at 1 unit/kg body wt. Glucose in tail vein blood was measured immediately before injection (time 0) and at 15, 30, 60, 90, and 120 min after injection using One-Touch Ultra glucometer (LifeScan, Milpitas, CA). At the end of the treatment period, the animals were killed by the intraperitoneal injection of 150 mg/kg sodium pentobarbital. Blood was collected via cardiac puncture, and serum was stored in aliquots at −80°C. For the mean arterial blood pressure (MAP) measurements, animals were placed under anesthesia (1–3% isoflurane), and arterial blood pressure was measured via the carotid artery by inserting a 27-gauge needle connected to a pressure transducer as described (40,41) followed by blood collection. While measurement of blood pressure under anesthesia may not recapitulate values in the conscious, unstressed state, we have previously reported that differences in MAP between experimental groups are maintained (40). Samples of visceral adipose tissue were collected on ice and rinsed in ice-cold PBS. For RNA isolation, tissue samples were preserved in RNAlater Solution (Applied Biosystems/Ambion, Austin, TX) at −80°C.

Quantitative real-time RT-PCR. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). Trace DNA was removed using a DNA-free kit (Applied Biosystems/Ambion, Austin, TX), and 0.5 μg of total RNA was converted to cDNA using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Quantitative real-time RT-PCR was performed using SsoFast EvaGreen Supermix (Bio-Rad) with primers spanning two or more exons and optimized for real-time PCR, which were designed using Geneious Pro software (v. 4.8; Biomatters Ltd., Auckland, New Zealand) and Beacon Designer (v. 7.70; Premier Biosoft International, Palo Alto, CA). Sequences for all used primers are shown in Table 1. Real-time PCR was performed using CFX96 real-time PCR detection system as follows: 50°C for 2 min, then 95°C for 2 min, then 40 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. Reaction specificity was confirmed by *1*) electrophoresis of products in 2% agarose gel after real-time RT-PCR to check if bands of expected size were detected; *2*) melting curve analysis; and *3*) sequencing amplified fragments. Relative gene expression was analyzed by ΔΔC(t) method using the CFX Manager software (v. 1.6; Bio-Rad, Hercules, CA).

Immunohistochemistry. To assess the macrophage infiltration in the adipose tissue, samples of the visceral adipose tissue were collected in 10% buffered formalin, processed, embedded in paraffin, and sectioned. Macrophages were stained as described (42) with the F4/80 antibody from Invitrogen/Caltag Laboratories (Carlsbad, CA), secondary horseradish peroxidase–conjugated antibody, diaminobenzidine chromogen, and a hematoxylin counterstain.

Measurement of serum levels of MCP-1 and adiponectin. Levels of MCP-1 and adiponectin in the cell culture medium were measured using the multiplex mouse adipocyte LINCOplex kit (Linco Research, St. Charles, MO) and the Luminex 100 system (Luminex, Austin, TX). Serum levels of MCP-1 and adiponectin were measured with the Milliplex MAP mouse single-plex kit and the

TABLE 1
Primers for gene expression analysis by quantitative RT-PCR

Gene	GeneBank Accession Number	Forward (5′–3′)	Reverse (5′–3′)	Product length, bp
Mouse				
Adiponectin	AF304466	GGAACTTGTGCAGGTTGGAT	TCCTGTCATTCCAACATCTCC	162
MCP-1	NM_011333	CGGAACCAAATGAGATCAGAA	TGTGGAAAAGGTAGTGGATGC	126
GAPDH	BC083149	GGGTGTGAACCACGAGAAATA	AGTTGTCATGGATGACCTTGG	104
TNF-α	NM_013693	CCAGTCTGTGTCCTTCTA	ATCTTGTGTTTCTGAGTAGTT	93
XOR	NM_011723	TATGACCGCCTTCAGAAC	TATGCCTTCCACAGTTGT	102
β-Actin	NM_007393	GAGAGGTATCCTGACCCTGAAGTA	TGTTGAAGGTCTCAAACATGATCT	203
Human				
Adiponectin	NM_004797	AAGGAGATCCAGGTCTTATTG	CCCACACTGAATGCTGAG	151
MCP-1	NM_002982	TGTGCCTGCTGCTCATAG	CTTGCTGCTGGTGATTCTTC	150
XOR	NM_000379	TGGTGGATGCTGTGGAGGAGATG	AAGATGGCGAGAGGCTGACTGAG	86
β-Actin	NM_001101	CGTGCGTGACATTAAGGAGA	AGGAAGGAAGGCTGGAAGAG	177

bp, base pairs.

mouse serum adipokine Milliplex MAP kit (Millipore, Billerica, MA), correspondingly using the Luminex 100 system.

Uric acid and thiobarbituric acid reactive substance assays. Uric acid was measured by an enzymatic assay with urate oxidase (Diagnostic Chemicals Limited, Oxford, CT). Thiobarbituric acid reactive substances were measured with the assay from Cayman Chemical (Ann Arbor, MI).

Statistics. At least three independent experiments were performed in the case of in vitro studies, and/or 6–10 animals per group were used for the in vivo studies. Each experiment was performed in triplicate. Data were analyzed by one-way ANOVA followed by Fisher least significant test, unpaired Student *t* test, or Mann-Whitney *U* test, with a value of $P < 0.05$ considered to represent a significant difference. Comparisons between two values were performed by *t* test or *U* test. ANOVA was used to test differences among several means.

RESULTS

Effect of uric acid on mRNA expression for MCP-1 and adiponectin: dose-response and time course experiments. To test the effects of uric acid on the adipokine production in vitro, we used differentiated mouse 3T3-L1 adipocytes and human primary subcutaneous adipocytes. When differentiated mouse adipocytes were incubated in the presence of 5 or 15 mg/dL uric acid for 3 days, we observed a significant dose-dependent increase in the mRNA abundance for MCP-1 (Fig. 1*A*). Even the lower dose of 5 mg/dL was significant ($P < 0.05$, *U* test). A time course experiment showed that 15 mg/dL uric acid induced an increase in MCP-1 mRNA at day 3, continued to increase to day 7, and plateaued between days 7 and 14 (Fig. 1*B*). The mRNA expression for adiponectin, which was induced by in vitro adipocyte differentiation, was lowered by both 5 mg/dL and 15 mg/dL of uric acid (Fig. 1*C*). In time course studies, uric acid also induced a gradual decrease in the adiponectin mRNA in adipocytes starting from day 3 to day 14 (Fig. 1*D*). Uric acid produced a similar effect in human primary subcutaneous adipocytes. Treatment with uric acid at a concentration of 15 mg/dL for 7 days induced a dramatic increase in mRNA level for MCP-1 and a decrease in the mRNA abundance for adiponectin (Supplementary Fig. 1*A* and *B*). A concentration of 7.5 mg/dL, which is a urate concentration typical for mild hyperuricemia in modern humans (43), did not produce statistically significant effects in this experiment (Supplementary Fig. 1).

Urate-induced activation of MCP-1 production in mouse adipocytes is prevented by antioxidants and rosiglitazone. We showed previously (15) that uric acid induced NOX-dependent reactive oxygen species (ROS) production in 3T3-L1 adipocytes, which triggered redox-dependent activation of the proinflammatory signaling by protein kinase p38. Therefore, we tested the hypothesis that production of proinflammatory factors in adipocytes

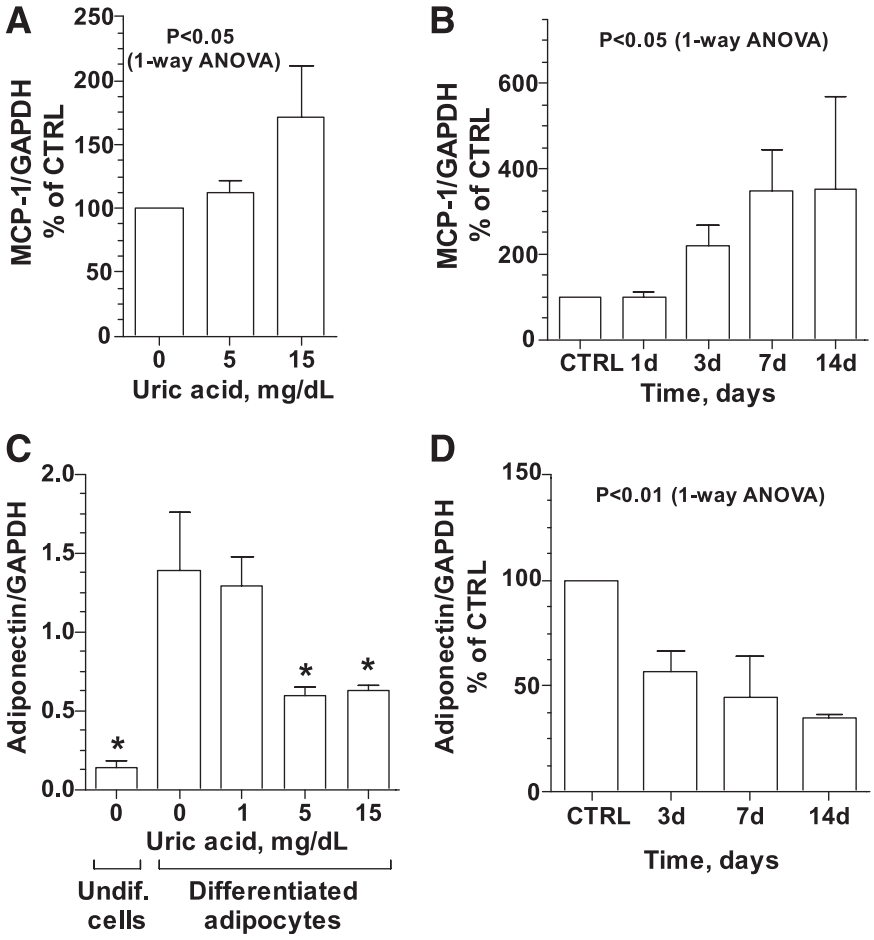

FIG. 1. Effect of uric acid on the mRNA expression for MCP-1 and adiponectin in 3T3-L1 adipocytes: time course and dose response. Differentiated 3T3-L1 adipocytes were treated with different concentrations of uric acid for varying periods of time. Relative mRNA expression for the MCP-1 (*A* and *B*) and adiponectin (*C* and *D*) in the dose response (*A* and *C*) and time course (*B* and *D*) for the effects of uric acid are shown. The values are mean ± SEM for 3–5 independent experiments performed at least in duplicate. *$P < 0.05$ (nonparametric Mann-Whitney *U* test) in comparison with untreated differentiated adipocytes. d, days; CTRL, control; undif., undifferentiated.

in response to uric acid is also redox-dependent, and NOX activity is required for this effect. 3T3-L1 adipocytes were treated with uric acid for 7 days with or without Mn(II) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP), a superoxide scavenger, or apocynin, an inhibitor of NADPH oxidase, and the MCP-1 and adiponectin mRNA and protein were determined. Uric acid induced a highly significant increase in MCP-1 mRNA in cells and protein concentration in the conditioned medium (Fig. 2*A* and *B*) while the presence of MnTMPyP or apocynin in the medium completely prevented these effects (Fig. 2*A* and *B*). These data suggest that urate-induced activation of the MCP-1 expression and secretion in adipocytes is mediated by the superoxide-dependent ROS, likely generated by NADPH oxidase. Moreover, urate-induced MCP-1 expression was abrogated by rosiglitazone, a PPAR-γ agonist (Fig. 2*C* and *D*), suggesting that activation of PPAR-γ prevents the effect of urate.

Urate-induced decrease in the production of adiponectin in mouse adipocytes is prevented by rosiglitazone but not antioxidants. Treatment of adipocytes with 15 mg/dL uric acid for 7 days induced a dramatic fall in the adiponectin mRNA level, which was accompanied by moderate but significant decrease in the adiponectin concentration in the incubation medium (Fig. 3*A* and *B*). In contrast to MCP-1, the effect of uric acid on adiponectin production was not prevented by either MnTMPyP or apocynin (Fig. 3*A* and *B*), while rosiglitazone restored adiponectin mRNA in cells and adiponectin levels in conditioned media to control levels (Fig. 3*C* and *D*). These experiments suggest that uric acid–induced inhibition of adiponectin production is not mediated by redox-dependent signaling but rather by a mechanism involving PPAR-γ.

Effect of uric acid on mRNA expression for XOR. PPAR-γ activation by rosiglitazone prevented the effects of uric acid in our experiments. On the other hand, XOR, an

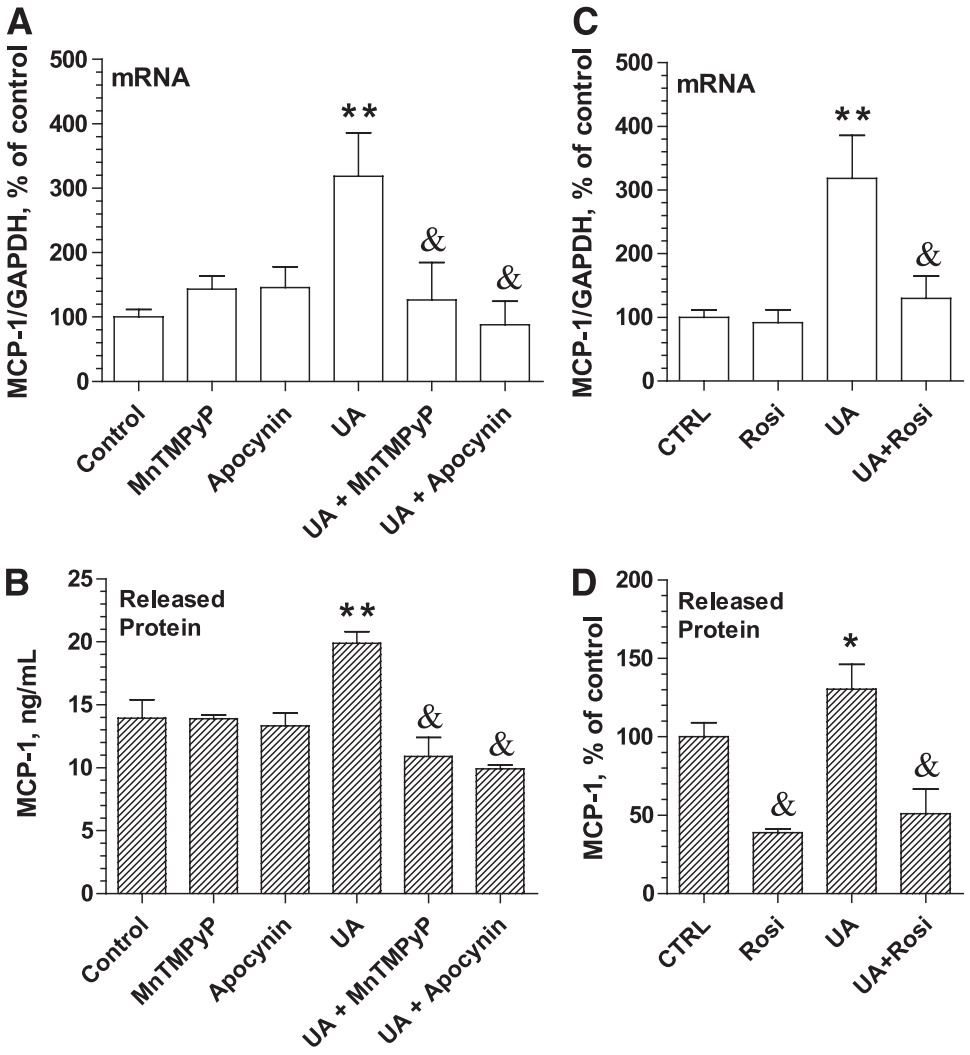

FIG. 2. Effect of the superoxide scavenger MnTMPyP and inhibitor of NADPH oxidase apocynin on the uric acid–induced increase in the mRNA expression and protein release for MCP-1 in 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated in the presence of 15 mg/dL uric acid with or without MnTMPyP (25 μmol/L, 30-min preincubation), apocynin (200 μmol/L), or rosiglitazone (10 μmol/L) for 7 days. Medium was changed once during this period with the fresh aliquot containing the same additives and stored at −80°C to pool with the medium collected at the end of the treatment. Total mRNA was isolated from the monolayer while media were used for measuring concentration of adipokines. The effect of uric acid in the presence or absence of antioxidants is shown in *A* for the relative expression of the mRNA for MCP-1 and in *B* for the concentration of MCP-1 in the pooled conditioned medium. The effect of rosiglitazone on the urate-stimulated MCP-1 production is shown in *C* (relative mRNA expression) and in *D* for the released protein. The values are mean ± SEM for three independent experiments performed in triplicate. *$P < 0.05$ and **$P < 0.01$ (nonparametric Mann-Whitney *U* test) in comparison with untreated adipocytes. &$P < 0.05$ (nonparametric Mann-Whitney *U* test) for the effect of an antioxidant/rosiglitazone. CTRL, control; Rosi, rosiglitazone; UA, uric acid.

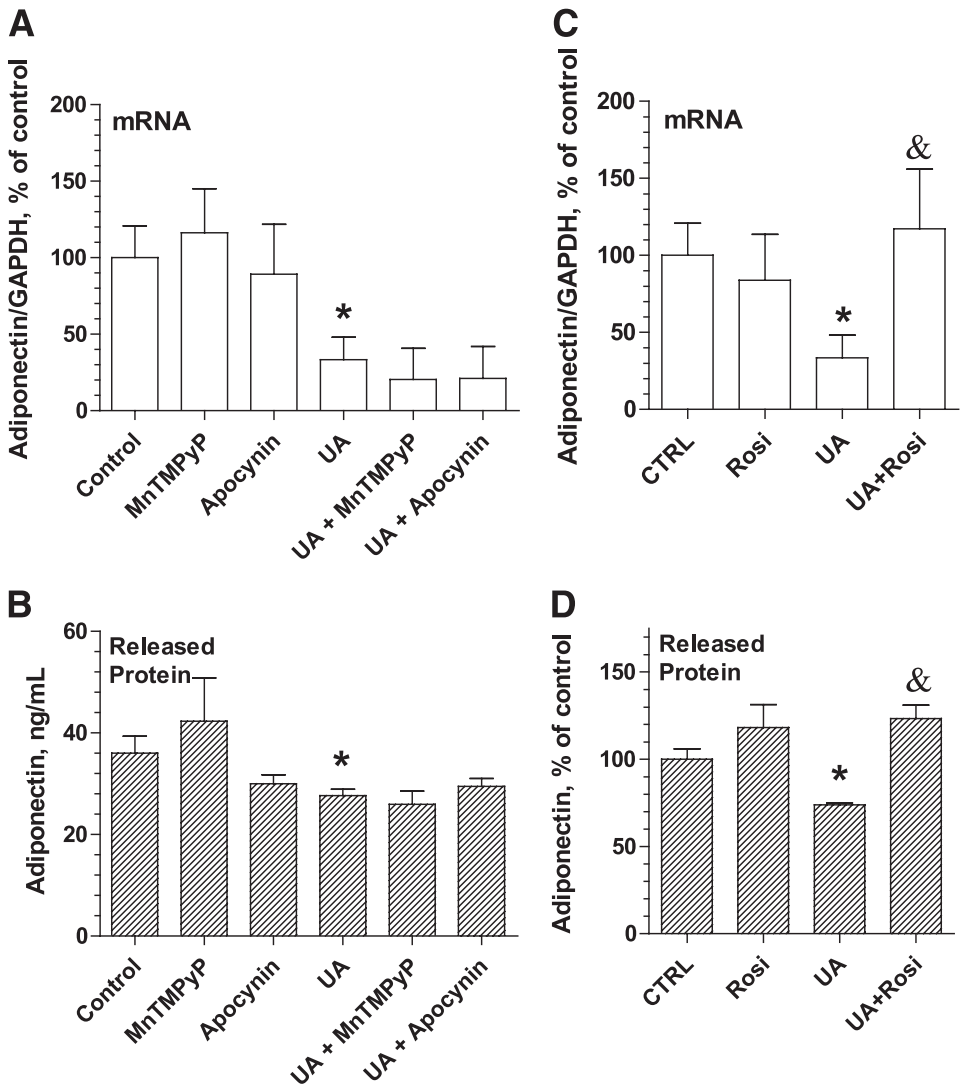

FIG. 3. Effect of the superoxide scavenger MnTMPyP and inhibitor of NADPH oxidase apocynin on the uric acid–induced decrease in the mRNA expression and protein release for adiponectin in 3T3-L1 adipocytes. The experimental conditions were as described in Fig. 2. The effect of uric acid in the presence or absence of antioxidants is shown in *A* for the relative expression of the mRNA for adiponectin and in *B* for the concentration of adiponectin in the pooled conditioned medium. The effect of rosiglitazone on the urate-stimulated adiponectin production is shown in *C* (relative mRNA expression) and *D* for the released protein. The values are mean ± SEM for three independent experiments performed in triplicate. **P* < 0.05 (nonparametric Mann-Whitney *U* test) in comparison with untreated adipocytes. &*P* < 0.05 (nonparametric Mann-Whitney *U* test) for the effect of an antioxidant/rosiglitazone. CTRL, control; Rosi, rosiglitazone; UA, uric acid.

enzyme-producing uric acid, is a crucial upstream regulator of PPAR-γ activity (38). Therefore, we hypothesized that uric acid could induce downregulation of PPAR-γ by affecting XOR via negative feedback mechanism. We tested an expression of the XOR mRNA in mouse and human adipocytes treated with uric acid for 7 days and found that uric acid induced a significant decrease in the XOR mRNA abundance in both cell types (Supplementary Fig. 2*A* and *B*).

Effect of lowering serum uric acid in mice with metabolic syndrome. We analyzed several animal models of obesity and metabolic syndrome or type 2 diabetes including Zucker diabetic fatty rats, ZSF1 rats, and the Pound mouse as well as corresponding lean control animals of the same age to compare the blood levels of uric acid in obese and lean animals. As shown in Fig. 4*A*, for each analyzed model, the level of uric acid in 8–week-old obese animals was substantially higher than in the corresponding lean animals. The greatest ratio between the level of uric acid in the lean control and obese animals was found in the Pound mice (Fig. 4*A*). These mice have metabolic syndrome but do not develop type 2 diabetes (39) and showed hyperuricemia compared with their littermates (Fig. 4*A*). Therefore, this model was selected for further experiments. To lower blood levels of uric acid, both lean and obese mice were treated with or without allopurinol for 8 weeks. Obese animals were hyperuricemic from the beginning of the study, and hyperuricemia progressed to even higher levels of uric acid during the course of the experiment (Fig. 4*B*). The correlation between the body weight and the level of uric acid in the Pound mouse is highly significant ($R = 0.860$, $N = 33$, $P < 0.001$). Serum uric acid substantially decreased in obese and lean animals after 2 weeks of treatments and remained low to the end of the study (Fig. 4*B*). The body weight gain was not affected by treatments with allopurinol in either obese (Fig. 4*C*) or lean animals (not shown).

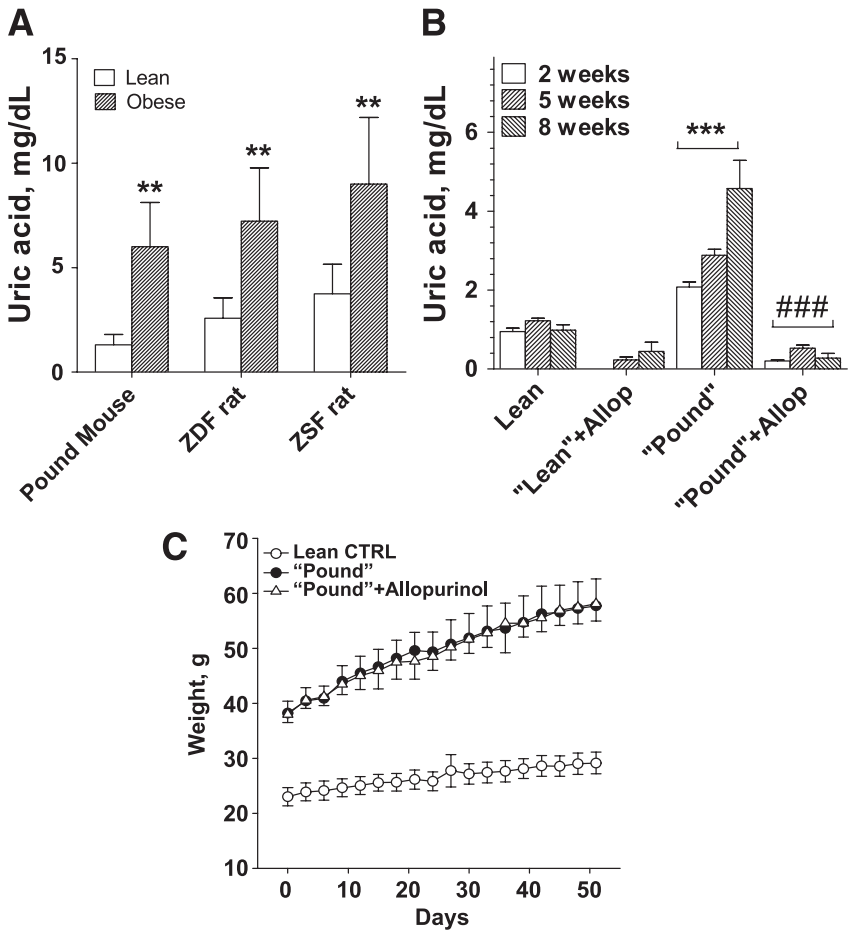

FIG. 4. Hyperuricemia in the mouse model of metabolic syndrome. *A*: Blood levels of uric acid in several models of obesity, metabolic syndrome, and diabetes: the Pound mouse (obesity, metabolic syndrome, described in the RESEARCH DESIGN AND METHODS section) vs. lean (+/?) control; ZDF *fa/fa* rats (obesity, type 2 diabetes) vs. lean (+/?) control; ZSF obese vs. lean (+/?) control. *B*: Changes in the blood levels of uric acid in the lean and obese Pound mice during the course of the experiment and the effect of treatments with allopurinol. Allopurinol did not affect body weight in the lean animals (not shown). *C*: Time course for the body weight for lean and obese Pound mice treated with allopurinol. The values are mean ± SEM (N = 8–10, performed in duplicate). **$P < 0.01$ and ***$P < 0.001$ (U test) for the effect of obesity. ###$P < 0.001$ (U test) for the effect of allopurinol. Allop, allopurinol; CTRL, control.

As expected, MCP-1 mRNA was about 10-fold higher in the adipose tissue of obese animals compared with lean control animals whereas adiponectin mRNA expression was about 20-fold lower than that observed in lean animals (Fig. 5*A* and *C*). Serum concentrations of MCP-1 and adiponectin protein changed correspondingly (Fig. 5*B* and *D*). Lowering uric acid with allopurinol reduced the MCP-1 mRNA expression and serum levels in the Pound mice by >50% while inducing a threefold increase in adiponectin mRNA level with an increase in the level of the circulating adiponectin. Thus, hyperuricemia in the obese mice with metabolic syndrome might be partially responsible for the proinflammatory generation of MCP-1 by adipose. In addition, elevated levels of uric acid might contribute to the decrease in adiponectin. Because lowering urate blood levels with allopurinol improved the balance of the production of MCP-1 and adiponectin, hyperuricemia might contribute to the proinflammatory endocrine imbalance in the adipose tissue.

In order to check whether lowering uric acid reduced signs of inflammation in the adipose tissue in addition to MCP-1 production, we examined the visceral adipose tissue for the presence of macrophages, a key step in proinflammatory changes in obesity (42,44). The number of the F4/80-stained macrophages was negligent in lean mice and increased dramatically in obese mice (Fig. 6*A*, *B*, and *D*) while it significantly decreased in obese mice treated with allopurinol (Fig. 6*C* and *D*). Because expression of the proinflammatory cytokine TNF-α in the adipose tissue is elevated in obesity and it is macrophage-specific (44), we measured the abundance of mRNA for TNF-α in the adipose tissue. As expected, the TNF-α mRNA was induced dramatically in the Pound mice while allopurinol treatment reduced it (Fig. 6*E*), consistent with changes in macrophage infiltration and changes in the MCP-1 production. Obesity is associated also with oxidative stress in the adipose tissue (45). Proinflammatory effects of uric acid in our experiments were also redox-dependent. We assessed the effect of lowering uric acid on the level of oxidative stress by measuring malondialdehide (MDA), a product of lipid peroxidation. MDA levels in the visceral adipose tissue and serum of obese mice was increased while allopurinol treatment substantially reduced it (Fig. 6*F* and *G*).

Effect of lowering uric acid in Pound mice on signs of metabolic syndrome. The proinflammatory endocrine imbalance and low-grade inflammation in the visceral adipose tissue is an underlying mechanism in the insulin

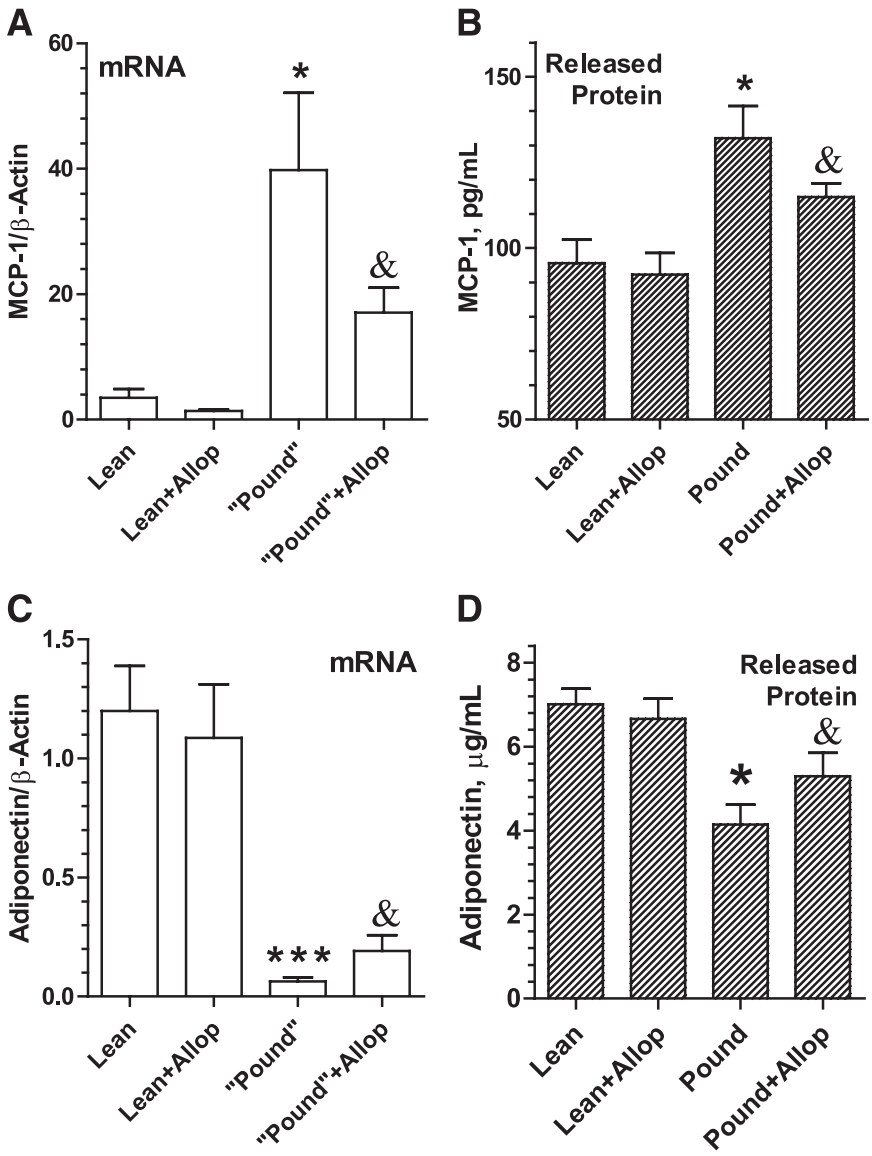

FIG. 5. The effect of lowering uric acid with allopurinol on the MCP-1 and adiponectin production in the adipose tissue of the obese Pound mice. Obese Pound mice and lean (+/?) control mice were treated with allopurinol for 8 weeks as described in the RESEARCH DESIGN AND METHODS section. mRNA abundance (*A* and *C*) as well as blood levels (*B* and *D*) for MCP-1 (*A* and *B*) and adiponectin (*C* and *D*) were measured at the end of the experiment. The values are mean ± SEM (N = 8–10, performed in duplicate). *$P < 0.05$ and ***$P < 0.001$ (U test), correspondingly, for the effect of obesity. &$P < 0.05$ for the effect of allopurinol.

resistance and cardiovascular abnormalities in subjects with metabolic syndrome (18,19,29,30). To test whether lowering uric acid can improve signs of metabolic syndrome, we performed insulin tolerance tests and measured blood pressure in obese mice treated with allopurinol. As expected, obese mice had dramatically reduced insulin sensitivity (Fig. 7*A*) and elevated MAP in comparison with lean mice (Fig. 7*B* and *C*). Lowering uric acid significantly improved both parameters (Fig. 7*A*–*C*).

DISCUSSION

We found that uric acid can increase expression and release of MCP-1 and reduce production of adiponectin in cultured adipocytes. We further showed that an increase in MCP-1 with a reduction in adiponectin occurs in both adipose tissue and serum of the obese, hyperuricemic Pound mice, and that lowering uric acid can attenuate these changes. Obesity is associated with an increase in MCP-1 production (27,28) and a decrease in the adiponectin production (19,46) in the adipose tissue. These changes contribute substantially to the obesity-related low-grade inflammation and metabolic syndrome, including insulin resistance and hypertension (17,25). A dramatic increase in macrophage infiltration with expression of the proinflammatory cytokine TNF-α was observed in visceral adipose tissue in obese mice and is consistent with reported results (42,44). Importantly, lowering uric acid caused a reduction in macrophage infiltration and TNF-α expression as well as improved insulin sensitivity and blood pressure. This study may provide a mechanism linking hyperuricemia, obesity, and metabolic syndrome that has been shown previously in clinical studies (4,5,12). The observation that lowering uric acid improved but did not reverse the changes in MCP-1 and adiponectin, inflammation, and insulin resistance in the Pound mouse is

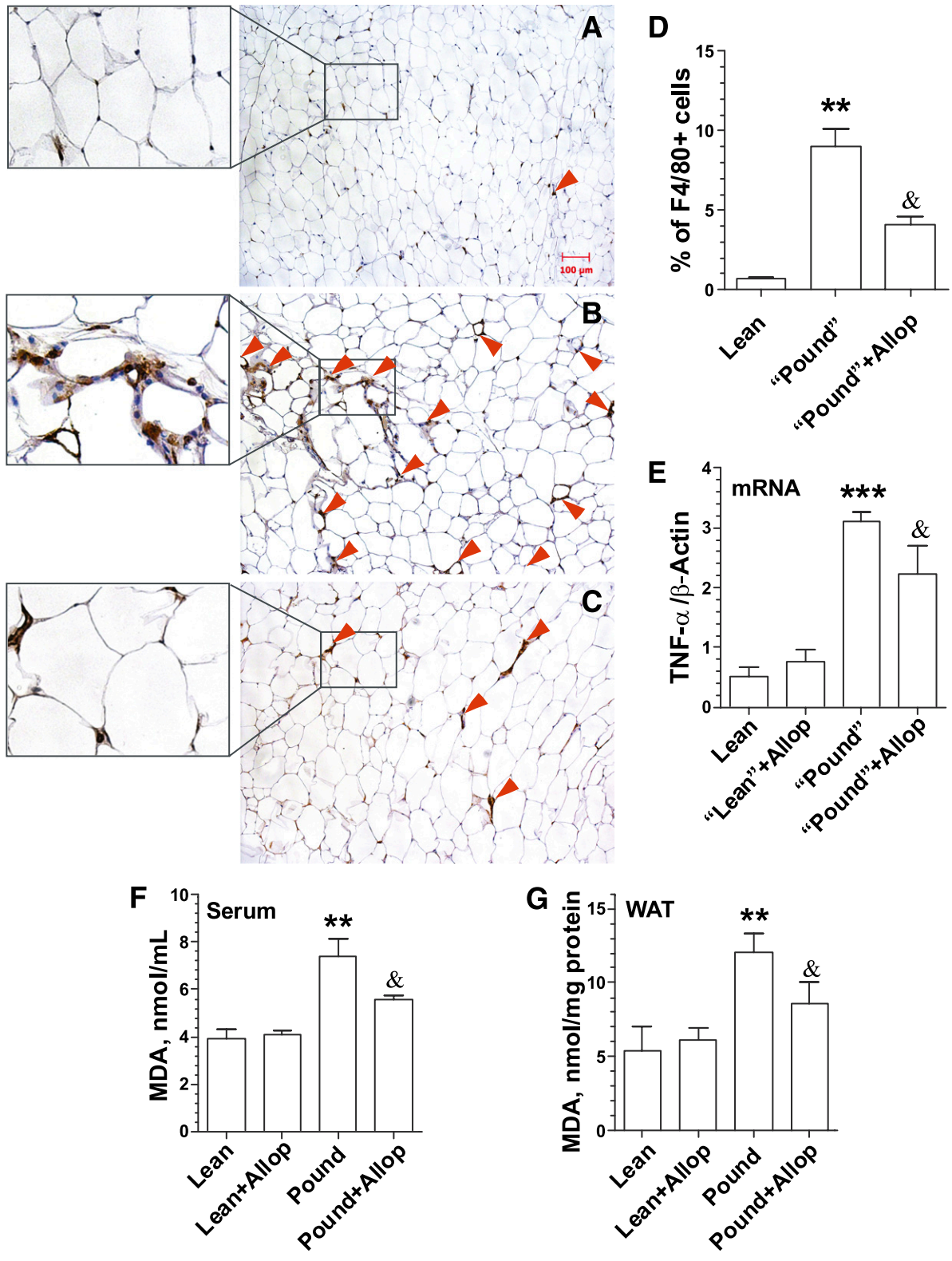

FIG. 6. The effect of lowering uric acid levels on the macrophage infiltration, TNF-α expression, and oxidative stress in the adipose tissue of the obese Pound mice. Samples of the adipose tissue from lean (*A*), obese (Pound) (*B*), and obese mice treated with allopurinol for 8 weeks (*C*) were stained with F4/80 antibody and counterstained with hematoxylin. Because diaminobenzidine was used as a chromogen, macrophages (F4/80-positive cells) are stained in brown as indicated with arrowheads (see also magnified rectangular region). *D*: The percentage of F4/80-positive macrophages within the adipose tissue is greatly induced by obesity and reduced by allopurinol treatment. Allopurinol did not affect macrophage staining in lean mice (not shown). Counting positive and negative cells were performed in a blind fashion at least in three fields per animal. *E*: Effect of allopurinol treatment on the relative expression of mRNA for TNF-α in the visceral adipose tissue of the obese (Pound) mice. In addition, the level of the oxidative stress in the serum (*F*) and in the visceral fat (*G*) in these mice was assessed by measuring the product of lipid peroxidation MDA using thiobarbituric acid reactive substance assay. The values are mean ± SEM (N = 5–6, performed in triplicate). $^{**}P < 0.01$ and $^{***}P < 0.001$ (*U* test), correspondingly, for the effect of obesity. &$P < 0.05$ for the effect of allopurinol. WAT, white adipose tissue. (A high-quality digital representation of this figure is available in the online issue.)

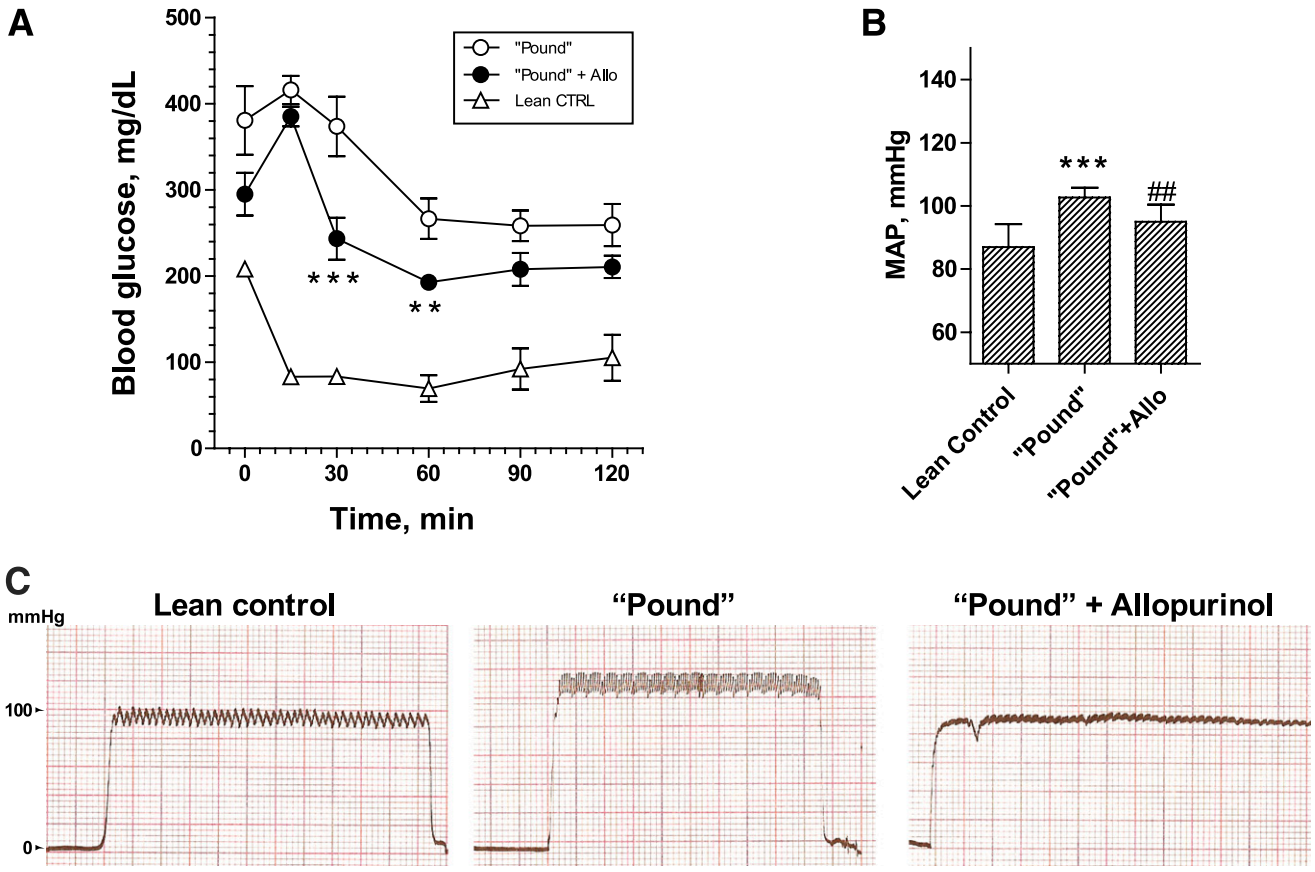

FIG. 7. Lowering uric acid levels in obese (Pound) mice improves signs of metabolic syndrome (insulin resistance, hypertension). *A*: To assess level of insulin sensitivity, the insulin tolerance test was used. Insulin (1 unit/kg) was injected, and blood level was measured at 0, 15, 30, 60, 90, and 120 min after injection. Sensitivity to insulin was normal in lean mice but dramatically reduced in obese mice, which was partially improved by treatment with allopurinol. *B*: Obesity induced increase in the MAP in mice, which was attenuated by allopurinol treatment. *C*: Representative recordings of blood pressure averaged in *B*. The values are mean ± SEM (*N* = 5–6, performed in triplicate). ***P* < 0.01 and ****P* < 0.001 (*U* test), correspondingly, for the effect of obesity. ##*P* < 0.01 for the effect of allopurinol. Allo, allopurinol; CTRL, control. (A high-quality color representation of this figure is available in the online issue.)

consistent with uric acid being a modifying factor—but not the sole causal factor—in driving these changes.

We observed an increase in serum uric acid in the Pound mice and other animal models of obesity in comparison with the corresponding lean controls. This observation is in agreement with published data (38,47). Hyperuricemia progressed as animals gained weight, and the level of uric acid correlated with the body weight. As these mice are obese and insulin-resistant because of the mutation in the leptin receptor (39), hyperuricemia cannot be considered as a causal factor of obesity in this case. However, an increase in uric acid contributed to the low-grade inflammation and metabolic syndrome via its direct effect on the production of MCP-1 and adiponectin in the adipose tissue.

In mouse 3T3-L1 adipocytes and human primary adipocytes, uric acid can induce a direct dose-dependent increase in the production of MCP-1 and a decrease in the production of adiponectin at the level of the mRNA expression and protein release. For mice, cells responded to uric acid beginning at concentrations of 5 mg/dL, which is similar to the levels of uric acid in the obese Pound mice. In the case of human adipocytes, cells responded to 7.5–15 mg/dL uric acid, which encompass the range observed in subjects with asymptomatic hyperuricemia to severe gout (3).

The ability of soluble uric acid to induce MCP-1 expression was first demonstrated in rat vascular smooth muscle cells (31). The effect of uric acid was mediated by MAP kinases ERK1/2 and p38 and nuclear factor-κB in a redox-dependent fashion (31). In our previous work with 3T3-L1 adipocytes (15), we showed that uric acid induced ROS production via activation of NADPH oxidase followed by phosphorylation of p38. The urate-induced increase in the MCP-1 expression in adipocytes was downregulated by a superoxide scavenger and a NOX inhibitor. Collectively, these data suggest that uric acid induced MCP-1 production in adipocytes via the redox-dependent signaling initiated by NOX activation. Furukawa et al. (45) reported that obesity was associated with oxidative stress in adipose tissue, which, in turn, caused an overexpression of a variety of proinflammatory cytokines including MCP-1. We observed an increase in oxidative stress in our mouse model of obesity by measuring MDA in the adipose tissue and serum. This was reduced by allopurinol, suggesting that hyperuricemia induced by obesity is another mechanism triggering oxidative stress followed by induction of the MCP-1 expression and inflammation in the adipose tissue.

The uric acid–induced increase in the MCP-1 production observed in adipocytes was prevented not only by antioxidants but also by activation of PPAR-γ with rosiglitazone. An activation of PPAR-γ is known to block the proinflammatory effects of TNF-α in adipocytes by affecting the proinflammatory branch of the nuclear

factor-κB–dependent signaling (48). XOR in adipocytes is thought to be a crucial upstream regulator of PPAR-γ activity (38). Treating adipocytes with exogenous uric acid reduced expression of XOR, which could be a cause of downregulated anti-inflammatory activity of PPAR-γ and facilitated redox-dependent MCP-1 production.

The mechanism of downregulation of the adiponectin production in obesity is not completely understood but appears to involve proinflammatory pathways (48,49), oxidative stress (45), and a deficiency in the PPAR-γ activity (48,50). The uric acid–induced decrease in adiponectin expression and secretion in our experiments was

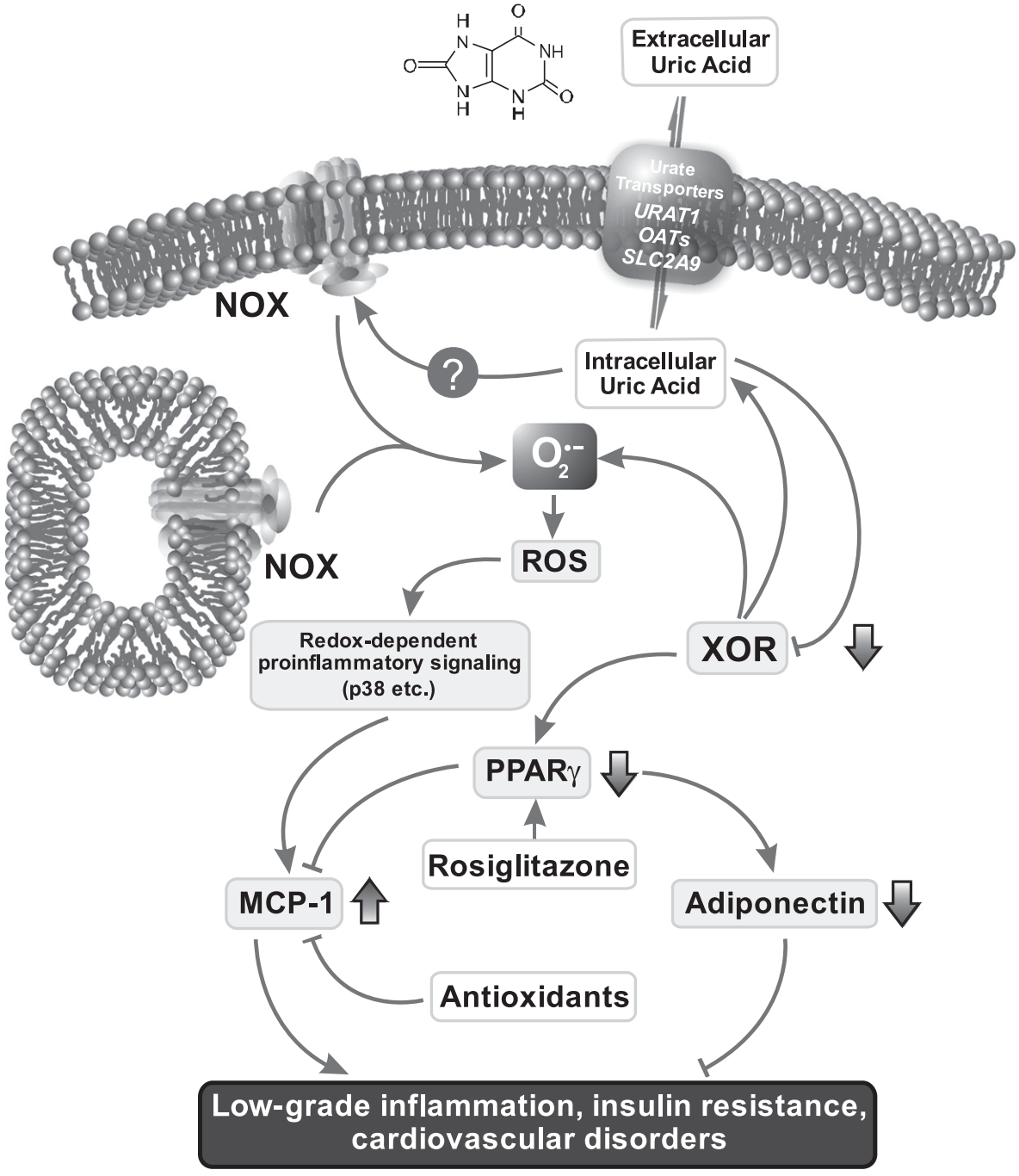

FIG. 8. Model for the effect of hyperuricemia on the endocrine balance in adipocytes. This model summarizes the results of the current and the previous (15) studies. Uric acid can enter adipocytes through a uric acid–specific transporter. We identified that adipocytes express at least one uric acid transporter, URAT1. Activation of NOX by uric acid occurs via unknown mechanism, and it was localized on the plasma membrane as well as on intracellular membranes. ROS generated from superoxide produced by NOX is followed by ROS-dependent activation of the proinflammatory signaling via p38. An activation of this mechanism in response to uric acid is followed by an increase in the production of MCP-1 and a decrease in the production of adiponectin. In addition, uric acid entering the adipocyte may downregulate expression of XOR, which (xanthine dehydrogenase but not xanthine oxidase) is known as a crucial upstream regulator of activity of PPAR-γ, a master-regulator of adipogenesis, expression of adiponectin, and an anti-inflammatory factor in adipocytes (38). Upregulation of MCP-1 in response to uric acid can be prevented by a superoxide scavenger or by inhibiting NOX. PPAR-γ activation can prevent both effects of uric acid. The effect of hyperuricemia might be partially responsible for the low-grade inflammation and insulin resistance in the adipose tissue and for increased risk of cardiovascular disease induced by obesity.

preventable by the PPAR-γ agonist, rosiglitazone, suggesting an involvement of the same kind of proinflammatory mechanism that was responsible for the induction of MCP-1. On the other hand, antioxidants could not improve adiponectin production affected by uric acid. We can suggest therefore that the deficiency of PPAR-γ but not oxidative stress was a primary trigger of this downregulation.

Because rosiglitazone prevented the uric acid–induced induction of MCP-1 and downregulation of adiponectin, these effects of uric acid might depend on PPAR-γ. XOR-dependent regulation of the adipocyte differentiation via control of PPAR-γ activity is known to be a finely orchestrated mechanism, which could be affected by manipulating the expression of XOR (38). Our data suggest that elevated concentrations of uric acid may reduce expression of XOR and attenuate PPAR-γ–dependent endocrine regulation in adipocytes.

In summary, uric acid can affect adipocytes directly by inducing effects resembling those observed in obesity: upregulation of proinflammatory factors and downregulation of the production of the insulin sensitizer and anti-inflammatory factor adiponectin via redox-dependent mechanisms, which can be prevented by an agonist of PPAR-γ (Fig. 8). In the mouse model of the metabolic syndrome, we observed hyperuricemia, which progressed with an increase in body weight. Lowering uric acid by inhibiting xanthine oxidoreductase in obese mice with the metabolic syndrome could improve the proinflammatory endocrine imbalance in the adipose tissue by lowering production of MCP-1 and increasing production of adiponectin.

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R.J.J. has several patent applications related to lowering uric acid as a means to treat hypertension and metabolic syndrome. He also has a lay book, *The Sugar Fix* (Rodale, 2008), that discusses the potential effects of fructose on obesity, diabetes, and cardiovascular disease.

No potential conflicts of interest relevant to this article were reported.

W.B. and S.M. researched data and reviewed and edited the manuscript. G.M., D.W., and V.P. researched data. C.B. contributed blood pressure measurements, contributed to discussion, and reviewed and edited the manuscript. R.J.J. contributed to discussion and reviewed and edited the manuscript. Y.Y.S. researched data and wrote the manuscript.

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REFERENCES

1. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. N Engl J Med 2008;359:1811–1821
2. Johnson RJ, Segal MS, Srinivas T, et al. Essential hypertension, progressive renal disease, and uric acid: a pathogenetic link? J Am Soc Nephrol 2005; 16:1909–1919
3. Johnson RJ, Perez-Pozo SE, Sautin YY, et al. Hypothesis: could excessive fructose intake and uric acid cause type 2 diabetes? Endocr Rev 2009;30: 96–116
4. Matsuura F, Yamashita S, Nakamura T, et al. Effect of visceral fat accumulation on uric acid metabolism in male obese subjects: visceral fat obesity is linked more closely to overproduction of uric acid than subcutaneous fat obesity. Metabolism 1998;47:929–933
5. Hikita M, Ohno I, Mori Y, Ichida K, Yokose T, Hosoya T. Relationship between hyperuricemia and body fat distribution. Intern Med 2007;46: 1353–1358
6. Zavaroni I, Mazza S, Fantuzzi M, et al. Changes in insulin and lipid metabolism in males with asymptomatic hyperuricaemia. J Intern Med 1993; 234:25–30
7. Moller DE, Kaufman KD. Metabolic syndrome: a clinical and molecular perspective. Annu Rev Med 2005;56:45–62
8. Reaven GM. The kidney: an unwilling accomplice in syndrome X. Am J Kidney Dis 1997;30:928–931
9. Fruehwald-Schultes B, Peters A, Kern W, Beyer J, Pfützner A. Serum leptin is associated with serum uric acid concentrations in humans. Metabolism 1999;48:677–680
10. Hayden MR, Tyagi SC. Uric acid: a new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: the urate redox shuttle. Nutr Metab (Lond) 2004;1:10
11. Masuo K, Kawaguchi H, Mikami H, Ogihara T, Tuck ML. Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. Hypertension 2003;42:474–480
12. Nakanishi N, Okamoto M, Yoshida H, Matsuo Y, Suzuki K, Tatara K. Serum uric acid and risk for development of hypertension and impaired fasting glucose or type II diabetes in Japanese male office workers. Eur J Epidemiol 2003;18:523–530
13. Yoo TW, Sung KC, Shin HS, et al. Relationship between serum uric acid concentration and insulin resistance and metabolic syndrome. Circ J 2005; 69:928–933
14. Cirillo P, Sato W, Reungjui S, et al. Uric acid, the metabolic syndrome, and renal disease. J Am Soc Nephrol 2006;17(Suppl. 3):S165–S168
15. Sautin YY, Nakagawa T, Zharikov S, Johnson RJ. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. Am J Physiol Cell Physiol 2007;293:C584–C596
16. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548–2556
17. Scherer PE. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 2006;55:1537–1545
18. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005;26:439–451
19. Matsuzawa Y. Adiponectin: identification, physiology and clinical relevance in metabolic and vascular disease. Atheroscler Suppl 2005;6:7–14
20. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev 2005;6:13–21
21. Goldstein BJ, Scalia R. Adiponectin: a novel adipokine linking adipocytes and vascular function. J Clin Endocrinol Metab 2004;89:2563–2568
22. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atheroslcerosis. Am J Physiol Heart Circ Physiol 2005;288:H2031–H2041
23. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circ Res 2005;96:939–949
24. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 2003;112:1785–1788
25. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005;115:1111–1119
26. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91
27. Kanda H, Tateya S, Tamori Y, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 2006;116:1494–1505
28. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci USA 2003;100:7265–7270
29. Peelman F, Waelput W, Iserentant H, et al. Leptin: linking adipocyte metabolism with cardiovascular and autoimmune diseases. Prog Lipid Res 2004;43:283–301
30. Hug C, Lodish HF. The role of the adipocyte hormone adiponectin in cardiovascular disease. Curr Opin Pharmacol 2005;5:129–134
31. Kanellis J, Watanabe S, Li JH, et al. Uric acid stimulates monocyte chemoattractant protein-1 production in vascular smooth muscle cells via mitogen-activated protein kinase and cyclooxygenase-2. Hypertension 2003;41:1287–1293

32. Kang DH, Park SK, Lee IK, Johnson RJ. Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. J Am Soc Nephrol 2005;16:3553–3562
33. Huang KC, Lue BH, Yen RF, et al. Plasma adiponectin levels and metabolic factors in nondiabetic adolescents. Obes Res 2004;12:119–124
34. Yamamoto Y, Hirose H, Saito I, et al. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. Clin Sci (Lond) 2002;103:137–142
35. Tamba S, Nishizawa H, Funahashi T, et al. Relationship between the serum uric acid level, visceral fat accumulation and serum adiponectin concentration in Japanese men. Intern Med 2008;47:1175–1180
36. Kono H, Rock KL. How dying cells alert the immune system to danger. Nat Rev Immunol 2008;8:279–289
37. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature 2003;425:516–521
38. Cheung KJ, Tzameli I, Pissios P, et al. Xanthine oxidoreductase is a regulator of adipogenesis and PPARgamma activity. Cell Metab 2007;5:115–128
39. Charles River Informational Resources. The C57BL/6NCrl-*Lepr*$^{db\text{-}lb}$/Crl Mouse: A Model for Metabolic Syndrome/Pre-Diabetes [Internet], 2008. Available from http://www.criver.com/SiteCollectionDocuments/rm_rm_r_POUND_MOUSE_fact_sheet.pdf. Accessed 10 April 2010
40. Erdely A, Wagner L, Muller V, Szabo A, Baylis C. Protection of wistar furth rats from chronic renal disease is associated with maintained renal nitric oxide synthase. J Am Soc Nephrol 2003;14:2526–2533
41. Muller V, Tain YL, Croker B, Baylis C. Chronic nitric oxide deficiency and progression of kidney disease after renal mass reduction in the C57Bl6 mouse. Am J Nephrol 2010;32:575–580
42. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112:1821–1830
43. Johnson RJ, Titte S, Cade JR, Rideout BA, Oliver WJ. Uric acid, evolution and primitive cultures. Semin Nephrol 2005;25:3–8
44. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–1808
45. Furukawa S, Fujita T, Shimabukuro M, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–1761
46. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784–1792
47. Kosugi T, Nakayama T, Heinig M, et al. Effect of lowering uric acid on renal disease in the type 2 diabetic db/db mice. Am J Physiol Renal Physiol 2009;297:F481–F488
48. Ruan H, Pownall HJ, Lodish HF. Troglitazone antagonizes tumor necrosis factor-alpha-induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NF-kappaB. J Biol Chem 2003;278:28181–28192
49. Liu M, Liu F. Transcriptional and post-translational regulation of adiponectin. Biochem J 2010;425:41–52
50. Tsuchida A, Yamauchi T, Takekawa S, et al. Peroxisome proliferator-activated receptor (PPAR)alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARalpha, PPARgamma, and their combination. Diabetes 2005;54:3358–3370