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 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). Adipokines 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
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, 0.25–15 µmol/L dexamethasone, and 0.5 mmol/L isobutyl-1-methylxanthine 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, 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 preclinical diabetes model (Charles River, Wilmington, MA). These mice have a mutation in the leptin receptor (a deletion of the exon 2), and diabetes/metabolic syndrome model (Charles River, Wilmington, MA). These mice were maintained 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 RNA later 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 CFX384 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) the electrophoresis of PCR 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 2-plex kit (Lincos Research, St. Louis, MO) and the Lumixin 100 system (Lumixin, Austin, TX). Serum levels of MCP-1 and adiponectin were measured using the Milliplex MAP Mice single-plex kit and the

| Gene      | Accession Number | Forward (5’–3’) | Reverse (5’–3’) | Product length, bp |
|-----------|------------------|-----------------|-----------------|-------------------|
| Mouse     |                  |                 |                 |                   |
| Adiponectin | AF304466         | GGAACCTTGGCAAGGTGGCAT | TCTGTCATTCCACACATCC | 162              |
| MCP-1     | NM_011333        | CGGAAACAAATGAGATCAGA | TGGTGAAGAGGTTAGTGTTAG | 126              |
| GAPDH     | BC083149         | GGGTGTACCAACGGACAAAT | AGTTTCATGGTATGACCTTG | 104              |
| TNF-α     | NM_013693        | CAGGCTGTCAGTCTCTCTTA | ATCTGGTGTTCCTGAGTTG | 93               |
| XOR       | NM_011723        | TATGACCCGGCTTCAAGAC | TATGACCTCCACAGTTG | 102              |
| β-Actin   | NM_007393        | GAGAGATTATCTCAGACCCCTGAAT | TGTGGAGGCTTCAACATGCT | 203              |
| Human     |                  |                 |                 |                   |
| Adiponectin | NM_004797        | AAGGAGATCAACGAGCTTATGG | CCCACACTGAATGCTCAG | 151              |
| MCP-1     | NM_029282        | TGGTCTGAGTCGCATAG  | CTTGCTGAGTGTCGTTTTCT | 150              |
| XOR       | NM_000379        | TGGTGGATGCTGTTGAGTAGT | AAGATGGGAGAGGTGCTCAG | 86               |
| β-Actin   | NM_001101        | CGTGCTGAGACATTAGAGGA | AGGAGGAGAGGCTGGAAG | 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. 1A). 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. 1B). 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. 1C). 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. 1D). 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. 1A 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.](https://diabetes.diabetesjournals.org/content/60/4/1259)
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. 2A and B) while the presence of MnTMPyP or apocynin in the medium completely prevented these effects (Fig. 2A 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. 2C 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. 3A and B). In contrast to MCP-1, the effect of uric acid on adiponectin production was not prevented by either MnTMPyP or apocynin (Fig. 3A and B), while rosiglitazone restored adiponectin mRNA in cells and adiponectin levels in conditioned media to control levels (Fig. 3C 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

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**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.
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. 2A 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. 4A, 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. 4A). These mice have metabolic syndrome but do not develop type 2 diabetes (39) and showed hyperuricemia compared with their littermates (Fig. 4A). 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. 4B). 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. 4B). The body weight gain was not affected by treatments with allopurinol in either obese (Fig. 4C) or lean animals (not shown).
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. 5A and C). Serum concentrations of MCP-1 and adiponectin protein changed correspondingly (Fig. 5B 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 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. 6E), 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 malondialdehyde (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. 6F 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 resistance of the obese. Uric acid levels were reduced by allopurinol treatment, and this was associated with a decrease in proinflammatory cytokine expression and an increase in adiponectin. These changes were consistent with an improvement in insulin sensitivity and a decrease in body weight. In addition, allopurinol treatment reduced oxidative stress in the adipose tissue, as evidenced by a decrease in malondialdehyde levels. These findings suggest that lowering uric acid may be a potential therapeutic strategy for the treatment of metabolic syndrome.
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. 7A) and elevated MAP in comparison with lean mice (Fig. 7B and C). Lowering uric acid significantly improved both parameters (Fig. 7A–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.)
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

**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.)
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...
preventable by the PPAR-γ agonist, rosiglitazone, suggesting the 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 down-regulation.

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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