*Original Article*

# Increase in Glucose-6-Phosphate Dehydrogenase in Adipocytes Stimulates Oxidative Stress and Inflammatory Signals

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**In adipocytes, oxidative stress and chronic inflammation are closely associated with metabolic disorders, including insulin resistance, obesity, cardiovascular disease, and type 2 diabetes. However, the molecular mechanisms underlying these metabolic disorders have not been thoroughly elucidated. In this report, we demonstrate that overexpression of glucose-6-phosphate dehydrogenase (G6PD) in adipocytes stimulates oxidative stress and inflammatory responses, thus affecting the neighboring macrophages. Adipogenic G6PD overexpression promotes the expression of pro-oxidative enzymes, including inducible nitric oxide synthase and NADPH oxidase, and the activation of nuclear factor-κB (NF-κB) signaling, which eventually leads to the dysregulation of adipocytokines and inflammatory signals. Furthermore, secretory factors from G6PD-overexpressing adipocytes stimulate macrophages to express more proinflammatory cytokines and to be recruited to the adipocytes; this would cause chronic inflammatory conditions in the adipose tissue of obesity. These effects of G6PD overexpression in adipocytes were abolished by pretreatment with NF-κB inhibitors or antioxidant drugs. Thus, we propose that a high level of G6PD in adipocytes may mediate the onset of metabolic disorders in obesity by increasing the oxidative stress and inflammatory signals. *Diabetes* 55:2939–2949, 2006**

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CCR2, chemokine (C-C motif) receptor 2; DCF-DA, 2,7-dichlorodihydrofluorescein diacetate; G6PD, glucose-6-phosphate dehydrogenase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; IκB, inhibitory κB; IKK-β, IκB kinase-β; IL, interleukin; iNOS, inducible nitric oxide synthase; MCP1, monocyte chemoattractant protein 1; NAC, *N*-acetyl-L-cysteine; NF, nuclear factor; PBST, PBS with Triton X-100; TNF, tumor necrosis factor; ROS, reactive oxygen species; SOD, superoxide dismutase.

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The increasing epidemic of obesity and metabolic disorders, including type 2 diabetes, hyperlipidemia, and atherosclerosis, is an important predictor of current health issues (1,2). Many recent studies have revealed that adipose tissue of obese subjects secretes high levels of various inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), resistin, and monocyte chemoattractant protein 1 (MCP1), which are closely associated with metabolic disorders (3–6). Furthermore, it has been suggested that the interactions between adipose tissue and macrophages in obese subjects are crucial in chronic inflammatory conditions in obesity-related metabolic disorders such as insulin resistance and atherosclerosis (5–7). Therefore, it is important to understand the molecular mechanisms or signaling cues that regulate the interactions between macrophages and adipose tissue.

Recently, it has been demonstrated that obesity-induced oxidative stress in adipose tissue results in an increase in inflammatory signals, dysregulation of adipocytokines, and insulin resistance (8,9). Although oxidative stress is closely associated with inflammatory signals and gene expression through nuclear factor-κB (NF-κB) signaling (10,11), it is not completely understood how obesity or metabolic disorder states could increase oxidative stress. One of the major sources of cellular reactive oxygen species (ROS) is mitochondria (12), and its dysfunction contributes to several pathological conditions, including vascular complications of diabetes, neurodegenerative diseases, and cellular senescence (13–17). Furthermore, hyperglycemia and lipotoxicity observed in obesity and its related disorders are associated with mitochondrial dysfunction and generation of oxidative stress (18,19). However, defined mechanisms of mitochondrial dysfunction in obesity-related metabolic disorders have not been clearly understood. Another source of cellular ROS is pro-oxidative enzymes such as NADPH oxidase and inducible nitric oxide (NO) synthase (iNOS), and their abnormal increase also stimulates oxidative stress (8,12,20). Oxidative stress is determined by a balance between the generation of ROS and the ROS-scavenging antioxidant system, regulated by the intracellular NAD(P)H-to-NAD(P)$^+$ ratio. Interestingly, NADPH is the central component of both pro- and antioxidant processes. NADPH serves as a cofactor for the reduction of GSSG (oxidized glutathione) to its reduced form GSH (glutathione) by glutathione reductase, which scavenges ROS. NADPH is also required for ROS produc-

tion by pro-oxidant enzymes such as iNOS and NADPH oxidase. Therefore, the intracellular NADPH-to-$NADP^+$ ratio appears to be essential for the determination of cellular redox potential.

Glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme of the pentose phosphate pathway, is a major intracellular source of NADPH generation. Previous reports have demonstrated that NADPH produced by G6PD is required for both the production of ROS, including superoxide anions and NO, and the elimination of these ROS via glutathione peroxidase and catalase in different cell types (21,22). Recently, we revealed that G6PD is highly expressed in the adipocytes of several obese animal models, and its overexpression in the adipocytes provoked the dysregulation of lipid metabolism and adipocytokine expression, resulting in insulin resistance (23). Because the levels of G6PD expression are enhanced specifically in the adipocytes of obese subjects, we were prompted to investigate whether G6PD overexpression in adipocytes affects oxidative stress, inflammatory signals, and macrophage gene expression, thus mediating metabolic disorders.

In this study, we have shown that increased G6PD expression in adipocytes promoted oxidative stress and NF-κB signaling, which are the key causative factors of chronic inflammation and insulin resistance in obesity and its related metabolic disorders. In addition, G6PD overexpression in adipocytes activated macrophage-specific gene expression and enhanced monocyte recruitment onto adipocytes, which is frequently observed in the adipose tissue of obese subjects. Taken together, we propose that the G6PD expression levels in adipose tissue may be a promising indicator of obesity and insulin resistance, and its regulation may be a novel therapeutic target in treating metabolic disorders.

## RESEARCH DESIGN AND METHODS

**Reagents and inhibitors.** DHEA (dehydroepiandrosterone) and lipopolysaccharide were purchased from Sigma. BAY11-7082, diphenyleneiodonium chloride, and *N*-acetyl-L-cysteine (NAC) were purchased from Calbiochem. hTNFα was purchased from R&D Systems.

**Cell culture.** 3T3-L1 preadipocytes were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum (Gibco BRL). At 2 days after confluence (day 0), differentiation of the 3T3-L1 cells was induced in DMEM containing 10% fetal bovine serum (FBS; Gibco BRL), methylisobutylxanthine (500 μmol/l), dexamethasone (1 μmol/l), and insulin (5 μg/ml) for 48 h. Then, culture medium was changed on alternate days with DMEM containing 10% FBS and insulin (5 μg/ml). Raw264 cells were maintained with DMEM containing 10% FBS, and THP-1 cells were maintained with RPMI containing 10% FBS.

**Fractionation of mouse adipose tissue and isolation of peritoneal macrophage.** Epididymal adipose tissues were excised from C57BL/6 and *db/db* mice (12 weeks old), rinsed in PBS, minced, and digested for 45 min at 37°C in Krebs-Ringer phosphate buffer (pH 7.4) with 2% BSA and type I collagenase (1.75 mg/ml). Digested tissue was filtered through a 250-μm nylon mesh to remove undigested tissue and centrifuged at 3,000 rpm for 5 min. The floating adipocyte fraction and stromal-vascular pellet were washed several times. Peritoneal macrophages were isolated from C57BL/6 mice. The mice were injected intraperitoneally with sterile fluid thioglycollate medium. After 4 days, the peritoneal cells were harvested and washed with PBS containing 3 mmol/l EDTA. Primary macrophages were cultured with DMEM containing 10% FBS to allow cell adherence. The nonadherent cells were removed by washing with PBS, and the adherent macrophages were refed with DMEM containing 10% FBS or adipocyte culture supernatants (conditioned media).

**Adenovirus for G6PD.** G6PD adenovirus was produced by Neurogenex (Seoul, Korea). G6PD cDNA was in-frame fused with Flag epitope tag in its $NH_2$ terminus, which was cloned into an adenoviral vector, pEntrBHRNX. For adenoviral infection, 3T3-L1 adipocytes (at day 6 after differentiation) were incubated with serum-free DMEM and various titers (10–30 multiplicity of infection) of adenovirus for 16 h at 37°C. Then, culture medium was replaced with fresh medium. Each experiment was performed at 48–72 h after viral infection.

**Quantitative RT-PCR.** Total RNA was isolated with Trizol (Invitrogen Life Technologies). cDNA was synthesized using a SuperScript first-strand synthesis system (Invitrogen Life Technologies). Quantitative real-time PCR was performed on a iCycler real-time PCR detection system (Bio-Rad), using SYBR Green I (BioWhittaker Molecular Applications), with each primer set shown in online appendix Table 1 (available at http://diabetes.diabetesjournals.org). The relative values of each mRNA were normalized with the levels of cyclophilin or GAPDH mRNA.

**Electrophoretic mobility shift assay.** Nuclear extracts from the 3T3-L1 adipocytes were isolated as described in a previous report, with minor modifications (24), and used for electrophoretic mobility shift assay. Target DNA sequences of NF-κB were used as probes; one strand is as follows: 5′-AGTTGAGGGGACTTTCCCAGGC-3′. Double-stranded oligonucleotides were end-labeled with [γ-$^{32}$P]ATP and T4 polynucleotide kinase. The nuclear extracts were mixed with radio-labeled probes (1 pmol per 30,000 cpm) in reaction buffer: 4 mmol/l Tris (pH 7.9), 23 mmol/l HEPES, 66 mmol/l NaCl, 5 mmol/l $MgCl_2$, 0.7 mmol/l EDTA, 1 mmol/l dithiothreitol, 14% (vol/vol) glycerol, and 4 μg poly(dI-dC). After incubation for 25 min at room temperature, the samples were loaded onto a native polyacrylamide gel (4%).

**Measurement of cellular nitrite, hydrogen peroxide, and ROS levels.** Nitrite was measured using the Griess reaction (25). Culture media (100 μl each) were collected and incubated with an equal volume of Griess reagent for 10 min at room temperature. The nitrite concentration was determined from the absorbance measured at 550 nm, using sodium nitrite as the standard. Hydrogen peroxide was measured using an Amplex Red Hydrogen peroxide assay kit (Molecular Probes) (8). Culture media (50 μl each) were collected and incubated with Amplex Red reagent for 30 min at room temperature. Hydrogen peroxide was determined by an Envision 2102 multilabel reader at 560 nm (PerkinElmer). Cellular ROS was measured using 2,7-dichlorodihydrofluorescein diacetate (DCF-DA) and luminol (Molecular Probes). 3T3-L1 adipocytes were washed with PBS and incubated in the dark for 30 min with DCF-DA (5 μmol/l). The fluorescence of DCF-DA was monitored by a fluorescence microscope (Olympus) and quantitated by an Envision 2102 multilabel reader at an excitation wavelength of 492 nm and emission wavelength of 530 nm. Luminol (50 μmol/l) was used for the quantitative measurement of the cellular ROS. Chemiluminescence of luminol was determined using a Berthold LB9501 luminometer for 1 min. As a positive control of ROS measurement, differentiated 3T3-L1 adipocytes were treated with $H_2O_2$ (100 μmol/l) simultaneously with DCF-DA or luminol, and increased DCF-DA fluorescence or chemiluminescence caused by $H_2O_2$ was determined.

**Immunocytochemistry.** 3T3-L1 cells were differentiated on glass coverslips and infected with the indicated adenovirus. After 2 days, cells were cocultured with THP-1 monocytes and nonadherent THP-1 cells removed by washing with PBS. The THP-1 cells adhered to the 3T3-L1 cells were fixed with 3.7% paraformaldehyde, permeabilized with 0.5% PBS with Triton X-100 (PBST), and incubated with 0.1% PBST containing 3% BSA for blocking. Subsequently, the cells were incubated with CD68 monoclonal antibody (DakoCytomation) at room temperature for 1 h and washed with 0.1% PBST. They were then incubated at room temperature with tetramethyl rhodamine isothiocyanate–conjugated secondary antibodies. The coverslips were rinsed and placed on a slide glass with mounting solution containing 4′,6-diamino-2-phenylindole. The cells were visualized using a fluorescence microscope (Olympus), and CD68-positive cells were quantitated using imaging analysis program (LSM510 version 3.5; Carl Zeiss).

**Separation of adipocytes and macrophages.** Cocultured 3T3-L1 adipocytes and Raw264 macrophage cells were separated following the manufacturer's protocol, using a CD11b MicroBeads system (MACS; Miltenyi Biotec). Briefly, cocultured cells were collected and washed twice with 10 ml washing buffer (PBS supplemented with 2 mmol/l EDTA and 0.5% BSA). Total cells ($<10^7$) were finally suspended in 100 μl washing buffer, mixed with 10 μl CD11b microbeads, and incubated at 12°C for 15 min. Then, cells were washed with 1 ml washing buffer. The cell pellet was suspended with 500 μl washing buffer and loaded in a large cell column. Adipocyte fractions were flow throughed with 500 μl washing buffer in a magnetic field, and macrophage fractions were flow throughed in a nonmagnetic field with 1 ml washing buffer, using the plunger supplied, after column washing three times with 500 μl washing buffer.

## RESULTS

**G6PD mRNA is increased under obese and diabetic conditions.** Previously, we reported that G6PD expression is increased in the adipose tissue of several obese and diabetic mouse models (23). However, the specific stimuli

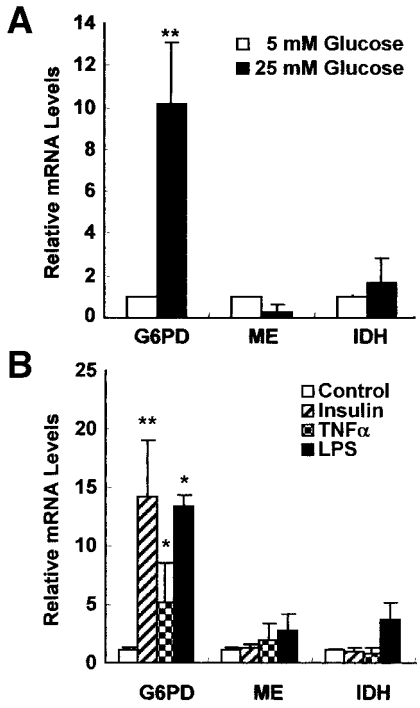


**FIG. 1. Induction of G6PD expression in 3T3-L1 adipocytes. *A*: mRNA expression of NADPH-producing enzymes, including G6PD, malic enzyme (ME), and isocitrate dehydrogenase (IDH), under high-glucose challenge. The 3T3-L1 adipocytes were incubated for 72 h with low glucose (5 mmol/l) or high glucose (25 mmol/l). **$P < 0.05$ vs. 5 mmol/l glucose by Student's *t* test. *B*: Induction of G6PD mRNA by insulin, cytokines, and endotoxin. After a 12-h starvation period, the cells were treated with high levels of insulin (5 μg/ml), TNF-α (10 ng/ml), and lipopolysaccharide (LPS; 1 μg/ml) for 6 h. *$P < 0.05$ vs. control; **$P < 0.01$ vs. control by Student's *t* test.**

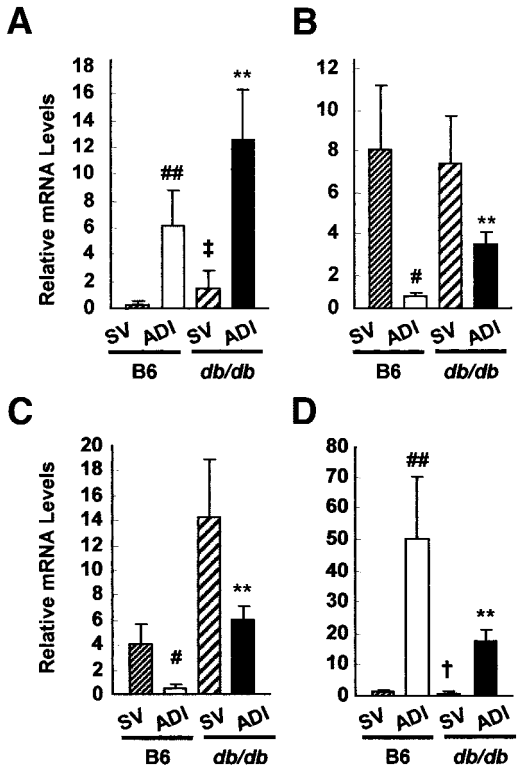


**FIG. 2. G6PD mRNA expression is enriched in primary adipocytes in fat tissues of C57BL/6 (B6) and *db/db* mice. *A*: The mRNA expression of G6PD in primary adipocytes and stromal-vascular fraction from fat tissues of lean (C57BL/6) and obese (*db/db*) mice. Control genes highly expressed in stromal-vascular fraction, MCP1 (*B*) and CD68 (*C*), were examined, and the adipocyte-specific gene adiponectin (*D*) was also examined. Results are the means ± SE. #$P < 0.05$ vs. C57BL/6 stromal-vascular fraction; ##$P < 0.001$ vs. C57BL/6 stromal-vascular fraction; †$P < 0.01$ vs. C57BL/6 stromal-vascular fraction; ‡$P < 0.001$ vs. C57BL/6 stromal-vascular fraction; **$P < 0.001$ vs. C57BL/6 adipocyte fraction by Student's *t* test. ADI, adipocyte fraction; SV, stromal-vascular fraction.**

that regulate G6PD expression in adipocytes under such conditions have not been identified. To investigate this, 3T3-L1 adipocytes were exposed to several conditions associated with metabolic disorders, and the levels of G6PD mRNA were examined by quantitative real-time PCR. As shown in Fig. 1*A*, G6PD mRNA expression was significantly increased by high-glucose treatment, whereas the other NADPH-producing enzymes, such as malic enzyme and isocitrate dehydrogenase, were only marginally affected. Similarly, high levels of insulin, TNF-α, and lipopolysaccharide remarkably elevated G6PD mRNA in adipocytes (Fig. 1*B*), implying that in metabolic disorders, G6PD expression is promoted in adipocytes.

**G6PD is predominantly expressed in primary adipocytes in fat tissues.** To determine the specific cell types in which G6PD is expressed in adipose tissue, we separated primary adipocytes and stromal-vascular cells from epididymal fat tissue and examined their G6PD mRNA levels. Compared with the stromal-vascular fraction, G6PD mRNA was abundantly expressed in the adipocyte fraction and was higher in *db/db* than C57BL/6 lean mice (Fig. 2*A*). In accordance with previous reports (3,5,6,26), stromal-vascular cells abundantly express MCP1 and CD68 (Fig. 2*B* and *C*), whereas the adipocyte fraction predominantly expresses adiponectin (Fig. 2*D*). In addition, mRNA levels of MCP1 and CD68 were increased in adipocytes of obese mice, whereas adiponectin was decreased (Fig. 2). These results clearly indicate that G6PD is primarily expressed in adipocytes in fat tissues.

**Overexpression of G6PD in adipocytes elevates pro-oxidative enzymes and oxidative stress.** Although oxidative stress is a major causative factor in the etiology of metabolic disorders, defined mechanisms by which obesity induced oxidative stress have not been clearly understood. Consistent with previous reports showing that differentiated adipocytes preserve high amounts of ROS (8), we observed that the activity of G6PD, a major adipogenic NADPH-producing enzyme, was positively correlated with the degree of differentiation and the levels of ROS during adipogenesis (online appendix Fig. 1). Nevertheless, the effect of G6PD overexpression in adipocytes on oxidative stress control has not been thoroughly examined. To address this issue, we investigated whether the overexpression of G6PD in adipocytes would affect pro- or antioxidative signaling. In the G6PD-overexpressing adipocytes infected with G6PD adenovirus (AdG6PD), the mRNA levels of pro-oxidative enzymes, including iNOS and NADPH oxidase components (gp91$^{phox}$, p22$^{phox}$, p67$^{phox}$, p47$^{phox}$, and p40$^{phox}$), were elevated, whereas those of the antioxidative enzymes, including superoxide dismutase (Cu,Zn-SOD) and GPx-1 (glutathione peroxidase), were slightly increased or not altered (Fig. 3*A*). Our observation that adipogenic G6PD overexpression was unlikely to have induced pro-oxidative gene expression resulted from the effect of G6PD on adipocyte differentiation because AdG6PD-infected adipocytes exhibited little

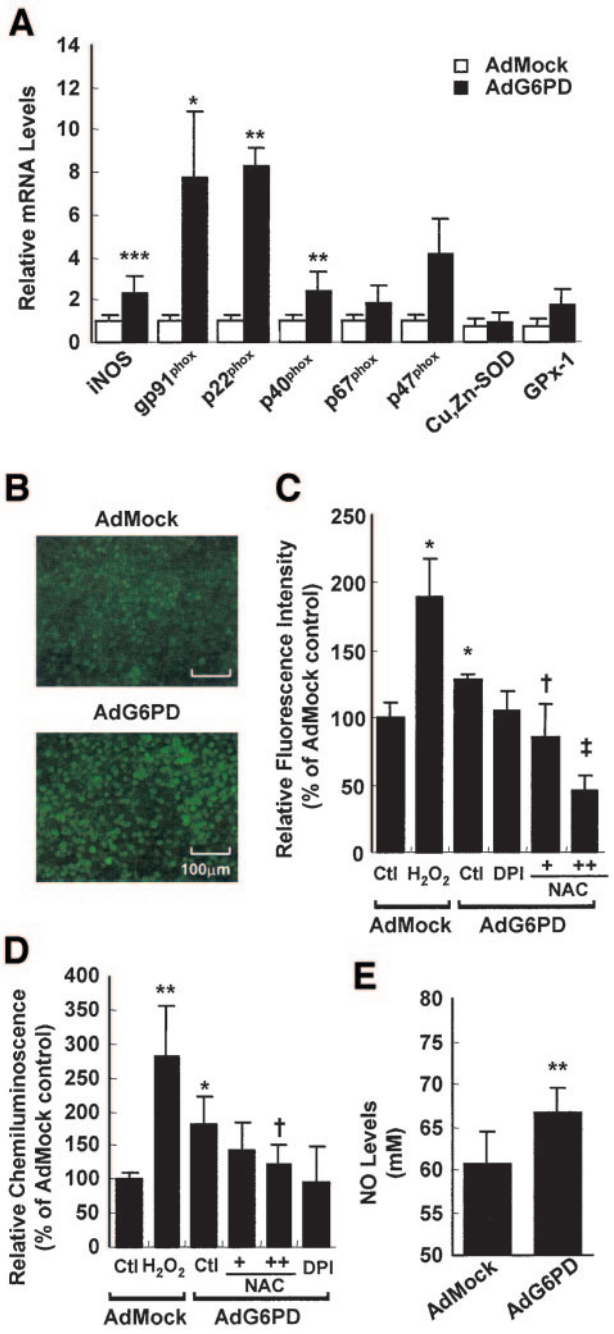


**FIG. 3. G6PD overexpression in adipocytes stimulates oxidative stress. *A*: mRNA expression of pro- and antioxidative enzymes by G6PD overexpression. The 3T3-L1 adipocytes were infected with AdMock or AdG6PD. Total RNA was isolated and analyzed by quantitative real-time PCR for iNOS, NADPH oxidase (gp91$^{phox}$, p22$^{phox}$, p40$^{phox}$, p67$^{phox}$, and p47$^{phox}$), Cu,Zn-SOD, and glutathione peroxidase-1 (GPx-1). Results are the means ± SE of three independent experiments performed in duplicate. *$P$ < 0.05 vs. AdMock; **$P$ < 0.01 vs. AdMock; ***$P$ < 0.001 vs. AdMock by Student's $t$ test. *B–D*: ROS accumulation in G6PD-overexpressing adipocytes. *B*: ROS production in AdMock- or AdG6PD-infected 3T3-L1 adipocytes was detected for 30 min with DCF-DA (5 μmol/l), which generates a fluorescent signal that is visualized by a fluorescence microscope. *C*: AdMock- or AdG6PD-infected 3T3-L1 adipocytes were treated with or without $H_2O_2$ (100 μmol/l), diphenyleneiodonium chloride (DPI; 10 μmol/l), 10 μmol/l NAC (+), and 10 mmol/l NAC (++). $H_2O_2$ was used as a positive control for ROS accumulation. Diphenyleneiodonium chloride and NAC were used as antioxidants. Intensities of DCF-DA fluorescence images were determined by multilabel reader. Results are the means ± SE of three independent experiments performed in triplicate. *$P$ < 0.01 vs. AdMock control; †$P$ < 0.01 vs. AdG6PD control; ‡$P$ < 0.001 vs. AdG6PD control by Student's $t$ test. *D*: Quantitative measurements of ROS accumulation in adipocytes were carried out by measuring the chemiluminescence of luminol. Results are normalized with protein concentration and are the means ± SE of two independent experiments performed in duplicate. **$P$ < 0.001 vs. AdMock control; *$P$ < 0.05 vs. AdMock control; †$P$ < 0.01 vs. AdG6PD control by Student's $t$ test. *E*: NO accumulation in G6PD-overexpressing adipocytes. Cultured media of AdMock- or AdG6PD-infected adipocytes were used for measurement of NO concentration. Results are the means ± SE of six independent experiments performed in triplicate. **$P$ < 0.001 vs. AdMock control by Student's $t$ test. Ctl, control.**

difference of adipogenic gene expression compared with mock adenovirus (AdMock)-infected adipocytes (online appendix Fig. 2). These results suggest that adipogenic G6PD overexpression promotes the pro-oxidative pathways rather than the antioxidative pathways, which might be linked to the increase of ROS production in the adipose tissue of obese subjects. To verify these observations, we directly determined the levels of cellular ROS and NO in G6PD-overexpressing adipocytes. Compared with AdMock controls, the AdG6PD-infected adipocytes exhibited increased cellular ROS (Fig. 3*B–D*) and NO levels (Fig. 3*E*). In parallel, we used TNF-α and hydrogen peroxide as positive controls for pro-oxidant signaling molecules (online appendix Fig. 3). TNF-α–treated adipocytes significantly induced expression of proinflammatory genes, such as IL-6 and MCP1, as well as pro-oxidative enzymes, such as iNOS and NADPH oxidase (gp91$^{phox}$). In contrast, hydrogen peroxide–treated 3T3-L1 cells did not significantly increase a certain set of genes, suggesting that hydrogen peroxide–induced oxidative stress might regulate a gene set slightly different from that of G6PD-dependent oxidative stress in adipocytes (online appendix Fig. 3). In addition, increased ROS level by G6PD overexpression was attenuated by an antioxidant, NAC, or an NADPH oxidase inhibitor, diphenyleneiodonium chloride (Fig. 3*C* and *D*). These data indicate that G6PD overexpression in adipocytes primarily contributes to the production of oxidative stress, which might induce proinflammatory response and insulin resistance.

**Increased G6PD expression in adipocytes stimulates NF-κB signaling.** It is well known that oxidative stress activates NF-κB signaling, which plays an essential role in insulin resistance and chronic inflammation in adipocytes (27–29). Because G6PD overexpression increased oxidative stress in adipocytes (Fig. 3), we examined whether G6PD overexpression in adipocytes would also alter NF-κB signaling and its target gene expression. To test this idea, 3T3-L1 adipocytes were infected with AdMock or AdG6PD and subjected to Western blot analyses with antibodies against p65, p50, inhibitory κB (IκB) kinase-β (IKK-β), and IκB-α. Compared with AdMock, the AdG6PD-infected adipocytes revealed increased expression of the p65 subunits of NF-κB in the nuclear fraction (Fig. 4*A*, *lane 3* vs. *lane 4*). TNF-α treatment was used as a positive control for the activation of NF-κB signaling (Fig. 4*A*, *lane 2*). In addition, the level of IκB-α, which is an inhibitor of NF-κB, was reduced, and that of IKK-β was 2.5-fold increased by G6PD overexpression compared with the AdMock controls (Fig. 4*B*, *lane 1* vs. *lane 3*). These effects were augmented by TNF-α treatment (Fig. 4*B*, *lane 4*). To confirm these results, the nuclear extracts of the 3T3-L1 adipocytes infected with either AdMock or AdG6PD were examined for the DNA-binding activity of NF-κB. In accordance with the above results, the DNA-binding activity of NF-κB was 1.8-fold increased in the G6PD-overexpressing adipocytes (Fig. 4*C*, *lane 1* vs. *lane 3*).

Because NF-κB signaling was stimulated in the G6PD-overexpressing adipocytes, we investigated the expression levels of NF-κB targets. As shown in Fig. 4*D*, iNOS was significantly elevated in the G6PD-overexpressing adipo-

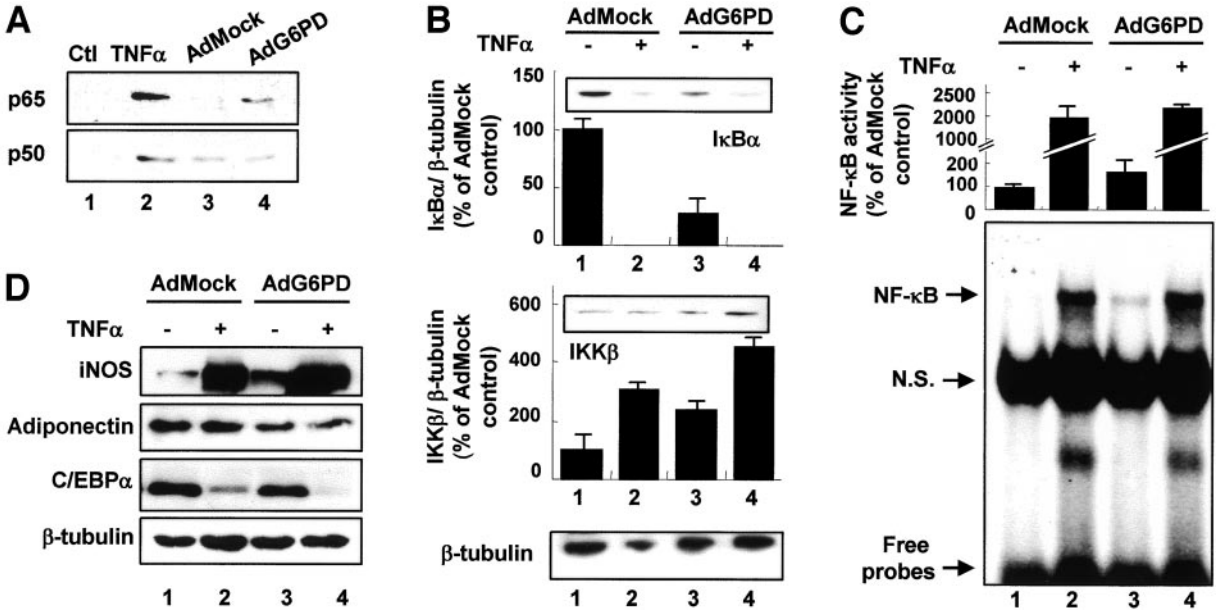


**FIG. 4. Adipogenic G6PD overexpression activates NF-κB signals. *A*: 3T3-L1 adipocytes were infected with AdMock or AdG6PD. Nuclear extracts were isolated and analyzed by immunoblotting with antibodies against p65 and p50. Adipocytes treated with TNF-α (5 ng/ml) were used as the positive control. *B–C*: 3T3-L1 adipocytes were infected with AdMock or AdG6PD. After infection, the cells were treated either with or without TNF-α (5 ng/ml) for 1 h. *B*: Cytosolic extracts of adipocytes were used for immunoblotting with antibodies against IκB-α, IKK-β, and β-tubulin. The intensity of each band is normalized with β-tubulin and is representative of the means ± SE of two independent experiments. *C*: Nuclear extracts were used for electrophoretic mobility shift assay to determine NF-κB activity. The intensity of each band is quantitated and is the means ± SE of two independent experiments. *D*: Regulation of NF-κB target gene expression in G6PD-overexpressing adipocytes. 3T3-L1 adipocytes were infected with AdMock or AdG6PD. The total cell lysates were subjected to immunoblotting with antibodies against iNOS, adiponectin, and CAAAT/enhancer-binding protein-α (C/EBPα). β-Tubulin was used as the loading control. Results are representative of three independent experiments. Ctl, control; N.S., nonspecific DNA-protein complex.**

cytes, whereas adiponectin and CAAAT/enhancer-binding protein-α were substantially decreased. Similar results were obtained on treatment with TNF-α (Fig. 4*D*, *lanes 2* and *4*). Taken together, these results imply that the oxidative stress produced by G6PD overexpression in adipocytes would, at least in part, contribute to insulin resistance and/or inflammatory signals by activating NF-κB.

**G6PD expression in adipocytes enhances proinflammatory gene expression.** Growing evidence suggests that oxidative stress in adipocytes is tightly correlated with inflammatory signals (8,9,30–32). The observation that G6PD overexpression in adipocytes enhanced oxidative stress prompted us to investigate the mRNA expression profiles of several proinflammatory genes, including TNF-α, IL-6, MCP1, resistin, and chemokine (C-C motif) receptor 2 (CCR2), that are associated with insulin resistance. In G6PD-overexpressing adipocytes, the expression of these proinflammatory genes was evidently increased (Fig. 5*A*). In addition, pretreatment with NF-κB inhibitors, such as BAY11-7082 and rosiglitazone, and the antioxidant NAC, as well as the G6PD inhibitor dehydroepiandrosterone (33), substantially reduced the effects of G6PD on proinflammatory gene expression (Fig. 5*A*). The effects of these drugs, including the NF-κB inhibitors, the antioxidant, and the G6PD inhibitor, also downregulated the expression of iNOS and NADPH oxidase in the G6PD-overexpressing adipocytes (Fig. 5*B*). Under the same conditions, mRNA levels of adiponectin and leptin were decreased by G6PD-overexpressing adipocytes (Fig. 5*C*). Collectively, these data suggest that increase of inflammatory signals in the G6PD-overexpressing adipocytes is mediated by oxidative stress and NF-κB activation.

**Adipogenic G6PD overexpression affects macrophage-specific gene expression.** Adipocytes secrete various adipocytokines that regulate whole-body energy homeostasis by acting on other tissues, including brain, liver, muscles, and macrophages (34). Recently, interactions between adipose tissue and peripheral macrophages have been implicated in the insulin resistance and inflammatory signals observed in obesity (3,6). However, the identity of the molecular linkers of this interaction were largely unknown. The observation that the increase in G6PD expression in adipocytes promoted oxidative stress and proinflammatory signals led to the examination of the effects of adipogenic G6PD overexpression on macrophage biology. To examine whether adipogenic G6PD overexpression alters humoral factors, we determined the levels of proinflammatory cytokines, such as MCP1, anti-inflammatory cytokines, such as adiponectin, and amounts of hydrogen peroxide from AdMock- or AdG6PD-infected adipocytes. In supernatants (conditioned media) from AdG6PD-infected adipocytes, the levels of secreted MCP1 and hydrogen peroxide were elevated, whereas that of adiponectin was decreased (Fig. 6*A* and *B*). Next, we investigated the effects of supernatants from G6PD-overexpressing adipocytes on primary cultured peritoneal macrophages. Peritoneal macrophages were incubated with supernatants from adipocytes infected with either AdMock or AdG6PD in the presence or absence of NAC, an antioxidant, or rosiglitazone, an antioxidant and anti-inflammatory drug (3,35,36) (Fig. 6*C*). Total RNA was isolated from each treated macrophage and analyzed for gene expression. Interestingly, the mRNA expression of macrophage genes, including iNOS, TNF-α, IL-6, MCP1, resistin, and CCR2, that are responsible for the inflamma-

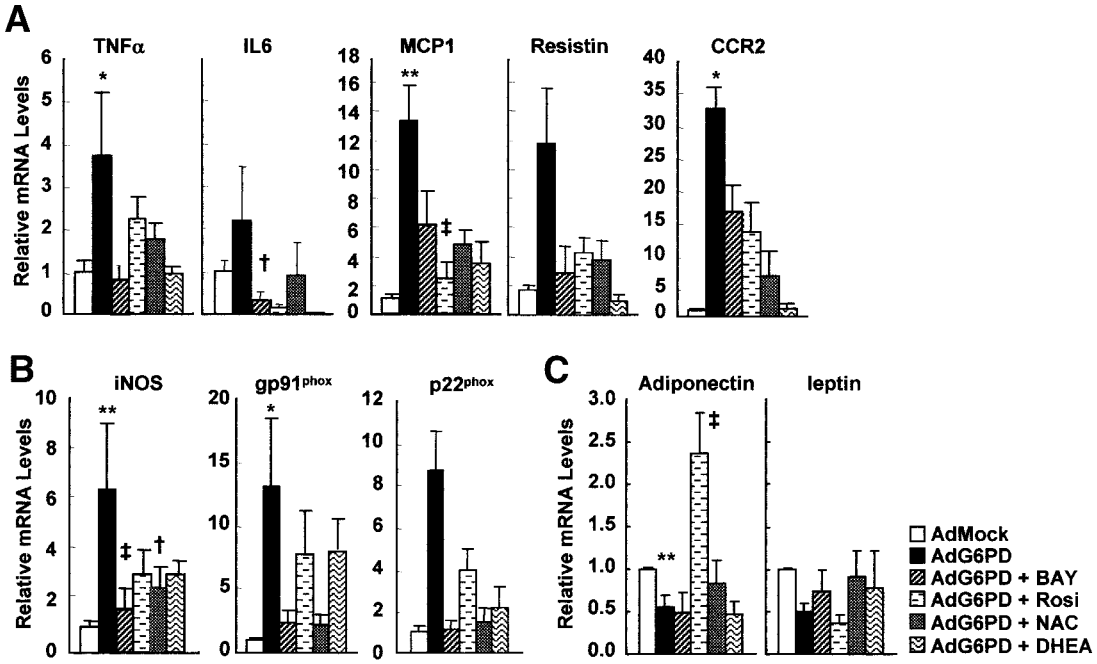


**FIG. 5. Effects of inhibitors of NF-κB and G6PD on the expression of inflammatory genes and pro-oxidative enzymes. 3T3-L1 adipocytes were infected with AdMock or AdG6PD. NF-κB inhibitors BAY11-7082 (3.3 μmol/l) and rosiglitazone (Rosi; 1 μmol/l), the antioxidant NAC (10 μmol/l), and the G6PD inhibitor dehydroepiandrosterone (DHEA; 100 μmol/l) were used to treat AdG6PD-infected 3T3-L1 adipocytes. Total RNA was isolated and analyzed for the expression of each mRNA by quantitative real-time PCR. mRNA levels of proinflammatory genes (*A*), including TNF-α, IL-6, MCP1, resistin, and CCR2, and those of pro-oxidative enzymes (*B*), such as iNOS and NADPH oxidase (gp91$^{phox}$ and p22$^{phox}$), were determined. *C*: In parallel, the mRNA levels of adiponectin and leptin were measured. Results are the means ± SE of two independent experiments performed in duplicate. *$P < 0.05$ vs. AdMock; **$P < 0.01$ vs. AdMock; †$P < 0.05$ vs. AdG6PD control; ‡$P < 0.01$ vs. AdG6PD control by Student's *t* test.**

tory signals and macrophage differentiation was enhanced by the supernatants from G6PD-overexpressing adipocytes (Fig. 6*D*). These effects were attenuated by NAC or rosiglitazone (Fig. 6*D*), indicating that certain molecules secreted from the G6PD-overexpressing adipocytes modulate proinflammatory signals, as well as macrophage-specific gene expression.

Recently, Suganami et al. (37) reported that adipocyte-derived mediators influence inflammatory gene expressions in macrophages, using a coculture system with adipocytes and macrophages. To examine the effects of adipogenic G6PD overexpression on the interaction between adipocytes and macrophages, macrophage Raw264 cells were cocultured with AdMock- or AdG6PD-infected 3T3-L1 adipocytes, and the inflammatory gene expressions were analyzed (Fig. 7*A*). As shown in Fig. 7*B*, expression of proinflammatory or macrophage-specific genes, including iNOS, TNF-α, IL-6, MCP1, CD11b, CD68, and SR-A, was remarkably increased when adipocytes were cocultured with macrophages, and these effects were augmented by G6PD overexpression in adipocytes. In addition, expression of cell adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, was enhanced by G6PD overexpression, suggesting that adipogenic G6PD increase would promote cross talk or physical interaction between adipocytes and macrophages, accompanied by modification of gene expression. Next, because inflammatory signaling molecules, such as MCP1, TNF-α, IL-6, and CCR2, are expressed in both adipocytes and macrophages, we decided to investigate the relative contribution of each cell type on inflammatory gene expression. Cocultured adipocytes and Raw264 cells were separated by CD11b microbeads (as detailed in RESEARCH DESIGN AND METHODS), and mRNAs from each cell type were isolated and analyzed (Fig. 7*C*). Interestingly, mRNA expression of MCP1 and IL-6 in both 3T3-L1 adipocytes and Raw264 macrophages was profoundly increased by coculture of adipocytes and macrophages and even further augmented by G6PD overexpression in adipocytes (Fig. 7*D*, *left panel*). In the case of iNOS and TNF-α, the relative induction folds of these genes were elevated in both cell types by coculture (Fig. 7*D*, *middle panel*). Although Raw264 cells predominantly expressed iNOS and TNF-α mRNA, induction of these genes was more remarkably increased in G6PD-overexpressing adipocytes by coculture (Fig. 7*D*, *middle panel*). In contrast, macrophage-specific genes, including CD68, CCR2, and CD11b, were elevated in Raw264 cells by coculturing with adipocytes, and they were slightly more increased by adipogenic G6PD overexpression (Fig. 7*D*, *right panel*). Additionally, the expression of intercellular adhesion molecule-1 in Raw264 cells and vascular cell adhesion molecule-1 in both Raw264 and 3T3-L1 cells was further enhanced by G6PD overexpression, suggesting that adipogenic G6PD might stimulate the interaction between macrophages and adipocytes. These results suggest that cross talk between adipocytes and macrophages promotes inflammatory signals and that the increase of inflammatory gene expression might be differently induced in a cell type–specific manner. Therefore, it is likely that adipogenic G6PD overexpression would activate the cross talk between adipocytes and macrophages and mediate enhanced chronic inflammatory signals in fat tissue in obesity.

**Adipogenic G6PD overexpression promotes the recruitment of THP-1 monocytes onto adipocytes.** Next, we investigated whether G6PD overexpression in adipo-

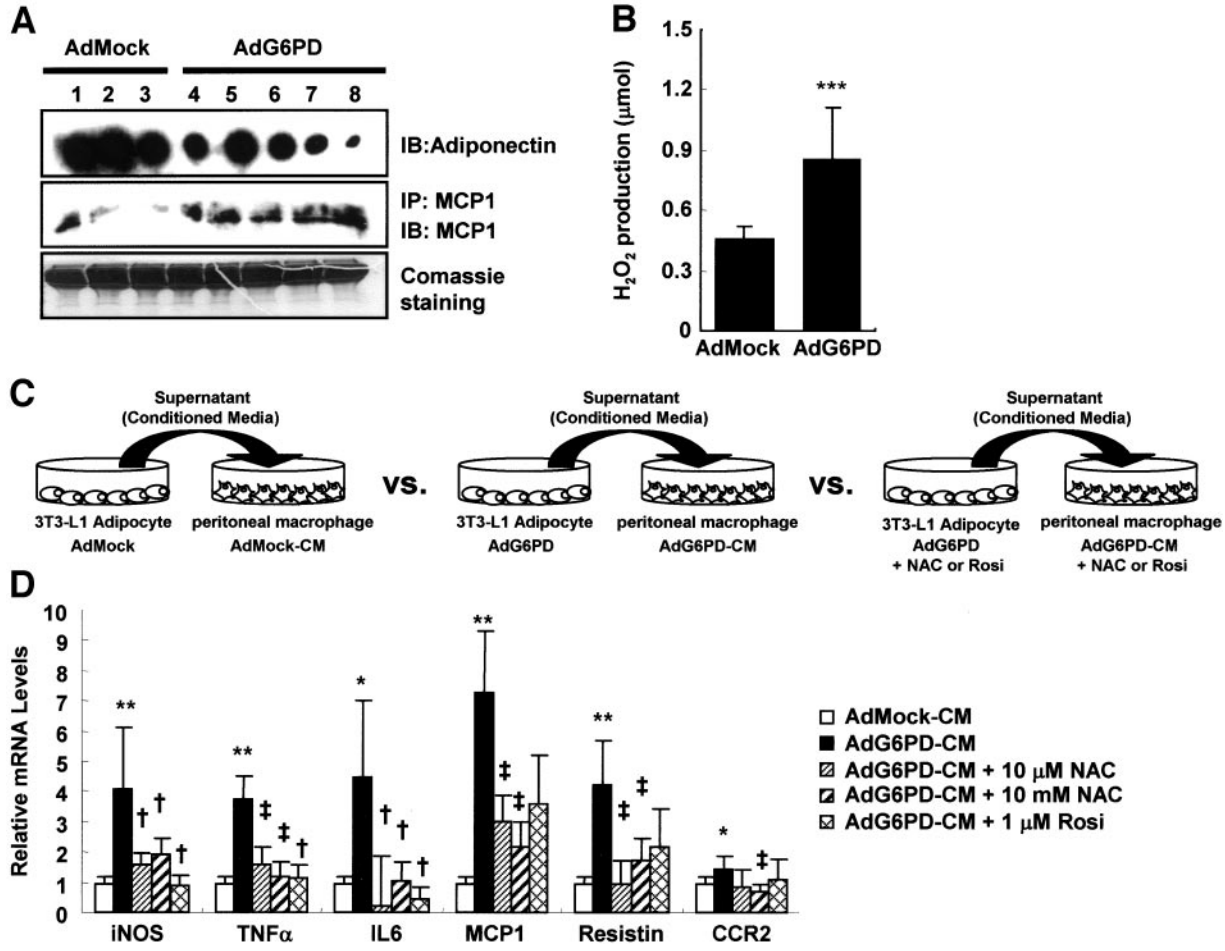


**FIG. 6. Conditioned media from G6PD-overexpressing adipocytes affect macrophage gene expression involved in chronic inflammation. *A*: Supernatants from control or G6PD-overexpressing adipocytes were harvested and analyzed by direct immunoblotting with antibodies against adiponectin. For MCP1, supernatants were immunoprecipitated with MCP1 antibody and analyzed by immunoblotting with MCP1 antibody. *B*: The levels of $H_2O_2$ in conditioned media from control or G6PD-overexpressing adipocytes were measured. ***$P < 0.001$ vs. AdMock control by Student's *t* test. *C*: Supernatants from control or G6PD-overexpressing adipocytes treated with or without NAC (10 μmol/l and 10 mmol/l) or rosiglitazone (Rosi; 1 μmol/l) were harvested and used to treat primary peritoneal macrophages of C57BL/6 mice. *D*: The relative amounts of each mRNA for iNOS, TNF-α, IL-6, MCP1, resistin, and CCR2 were analyzed by quantitative real-time PCR. Results are the means ± SE of two independent experiments performed in duplicate. *$P < 0.01$ vs. AdMock-conditioned media (CM); **$P < 0.001$ vs. AdMock-conditioned media; †$P < 0.01$ vs. AdG6PD-conditioned media; ‡$P < 0.001$ vs. AdG6PD-conditioned media by Student's *t* test.**

cytes promotes the direct recruitment of monocytes onto adipose tissue. To address this issue, AdMock- and AdG6PD-infected 3T3-L1 cells were incubated with human monocyte THP-1 cells. Consistent with the results above, we observed that more THP-1 cells were recruited to the G6PD-overexpressing adipocytes compared with the control adipocytes, and antioxidant NAC pretreatment partially attenuated monocyte recruitment (Fig. 8). These results suggest that G6PD-overexpressing adipocytes provide a certain milieu that might promote the recruitment of circulating monocytes onto adipocytes and induce macrophage infiltration into the adipose tissue of obese subjects.

## DISCUSSION

In obese subjects, adipose tissue exhibits increased oxidative stress, which is a major contributor to metabolic disorders such as insulin resistance, type 2 diabetes, cardiovascular disease, and atherosclerosis (8,31,38). It has been reported that antioxidant drugs, including α-lipoic acid and mimetics of SOD or catalase, improve diabetes complications by reducing oxidative stress (31,39,40). In addition, several drugs for cardiovascular diseases or type 2 diabetes, such as statins and thiazolidinediones, also decrease intracellular ROS (36,41,42). Thus, it is very likely that obesity-related oxidative stress is closely associated with metabolic disorders. Despite this, the question of how obesity produces oxidative stress remains to be elucidated.

Insulin resistance, a crucial factor for the development of metabolic disorders, is induced by several molecules, including insulin, free fatty acids, endotoxins, and certain cytokines such as TNF-α, IL-6, and MCP1, as well as by high levels of glucose. We found that the expression of G6PD was elevated in response to these types of stimuli; all of which are elevated in obesity and type 2 diabetes (Fig. 1). Moreover, among several NADPH-producing enzymes, G6PD in adipocytes was notably upregulated by these signals. Because these signals are also known to activate NF-κB signaling, resulting in the induction of inflammation and insulin resistance, we examined whether the upregulation of G6PD under these conditions was mediated by the NF-κB pathway. Of note, we observed that the mouse G6PD promoter was transactivated by NF-κB (data not shown). Thus, it appears that the G6PD in adipocytes might be actively involved in the pathogenesis of metabolic disorders such as obesity and insulin resistance,

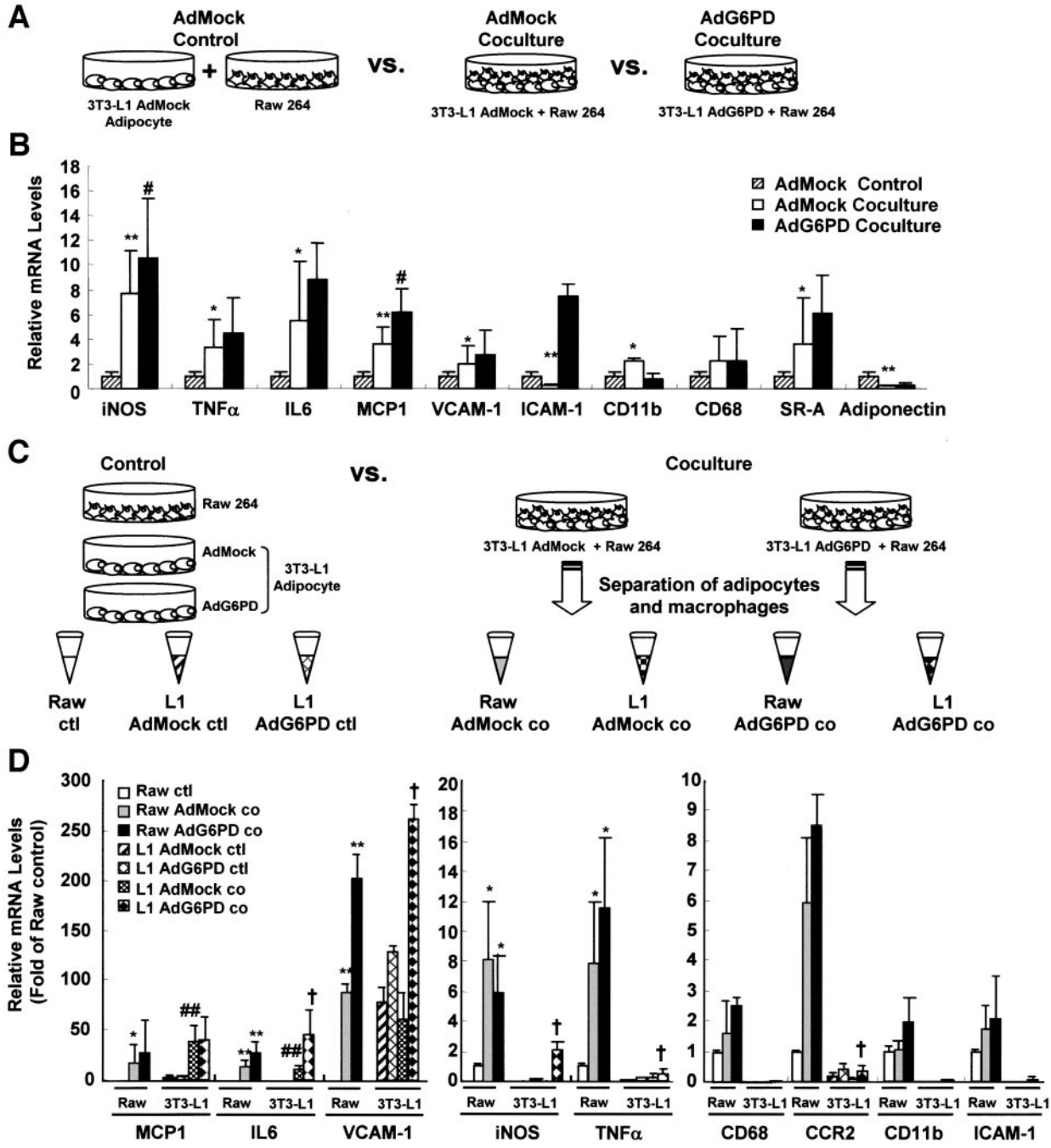


**FIG. 7. Inflammatory signals are increased by coculture with adipocytes and macrophages. *A*: Illustration of coculture system with adipocytes and macrophages. RAW264 cells were seeded onto AdMock- or AdG6PD-infected 3T3-L1 adipocytes. After 2 days of incubation, total RNA was isolated and analyzed. As control, equal numbers of adipocytes and macrophage cells were cultured separately and mixed after harvest. *B*: The relative amounts of each mRNA for iNOS, TNF-α, IL-6, MCP1, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), CD11b, CD68, SR-A, and adiponectin were analyzed by quantitative real-time PCR. Results are representative of the means ± SE of two independent experiments performed in duplicate. *$P < 0.01$ vs. AdMock control; **$P < 0.001$ vs. AdMock control; #$P < 0.01$ vs. AdMock coculture by Student's $t$ test. AdMock-infected adipocytes were separately cultured with Raw264 macrophage (AdMock control), AdMock-infected adipocytes were cocultured with Raw264 (AdMock coculture), and AdG6PD-infected adipocytes were cocultured with Raw264 (AdG6PD coculture). *C*: Illustration of coculture system and separation of adipocytes and macrophages after coculture. RAW264 cells were seeded onto AdMock- or AdG6PD-infected 3T3-L1 adipocytes. After 2 days of incubation, adipocytes and macrophages were separated by a CD11b microbead system (MACS; Miltenyi Biotec). Total RNA from each cell type was isolated and analyzed. As control, equal numbers of adipocytes and macrophages were cultured separately and used for analysis. *D*: The relative amounts of each mRNA for MCP1, IL-6, VCAM-1, iNOS, TNF-α, CD68, CCR2, CD11b, and ICAM-1 were analyzed by quantitative real-time PCR. Results are the means ± SE of two independent experiments performed in duplicate. *$P < 0.1$ vs. Raw264 control; **$P < 0.05$ vs. Raw264 control; #$P < 0.1$ vs. 3T3L1-AdMock coculture; ##$P < 0.05$ vs. 3T3L1-AdMock coculture; †$P < 0.1$ vs. 3T3L1-AdMock coculture by Student's $t$ test. co, coculture; ctl, control; Raw, Raw264.**

and the increase of G6PD in adipocytes might play a causative role in the development of metabolic disorders.

To further explore the molecular mechanism underlying G6PD-induced insulin resistance (23), we focused on oxidative stress and inflammatory signals because G6PD is a crucial enzyme to maintain cellular redox potential. In adipocytes, G6PD overexpression greatly enhances prooxidative enzymes, including iNOS and NADPH oxidase,

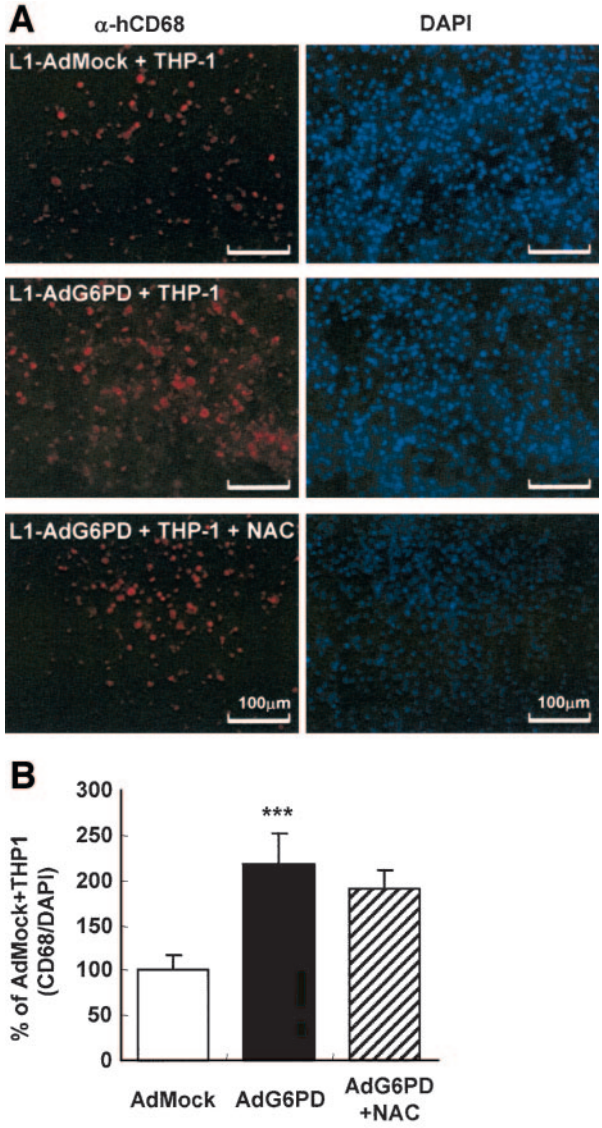


**FIG. 8. THP-1 monocyte recruitment onto adipocytes is enhanced by G6PD overexpression. *A*: THP-1 monocytes were cocultured with either AdMock- or AdG6PD-infected adipocytes for 2 days. Nonadherent monocytes were removed by washing, and the monocytes attached onto the adipocytes were immunostained with anti-CD68 antibody as described in RESEARCH DESIGN AND METHODS. *B*: Quantitative measurements of CD68-positive cells obtained by imaging analysis program (LSM510; Carl Zeiss) and normalized by 4′,6-diamino-2-phenylindole (DAPI). Results are representative of the means ± SE of five different images of two independent experiments. ***$P < 0.001$ vs. AdMock control by Student's *t* test.**

thus inducing oxidative stress (Fig. 3). Moreover, we believe that the G6PD-dependent increase of pro-oxidative enzymes is limited to adipocytes because G6PD overexpression in primary hepatocytes did not show any effects (online appendix Fig. 4). The elevated oxidative stress in the adipose tissue of obese subjects with high levels of NADPH oxidase activity (8) is closely correlated with the increase in G6PD expression (23). We also observed that the level of cellular NADPH in adipocytes was increased by G6PD overexpression, high glucose, high insulin, or TNF-α, which have been implicated in metabolic disorders (online appendix Fig. 5). In addition, it has been reported that the enzymatic activities of NADPH oxidase and iNOS, the major enzymes involved in oxidative stress, are elevated in obesity and metabolic disorders (8,20,43–46). Therefore, these findings strongly suggest that the abnormal increase of G6PD in the adipocytes of obese subjects leads to oxidative stress by upregulation of NADPH oxidase and iNOS. As mentioned above, mitochondria also play a crucial role in the generation of cellular ROS, and mitochondrial dysregulation has been implicated in metabolic disorders, including insulin resistance and diabetes (17,47–49). In this regard, the effects of adipogenic G6PD overexpression on mitochondrial function need to be further examined to get a better picture of metabolic disorders.

It has been demonstrated that oxidative stress–induced activation of NF-κB signaling appears to be associated with the pathogenesis of insulin resistance and type 2 diabetes (28,29,50,51). Many studies have also reported that antioxidant drugs and IKK-β inhibitors protect against insulin resistance in obesity and type 2 diabetes (27,31,51,52). Thus, it is of interest that G6PD-overexpressing adipocytes enhanced NF-κB expression and its DNA-binding activity (Fig. 4), accompanied by regulation of NF-κB target genes, including inflammatory and chemokine genes (Fig. 5). Consequently, the G6PD-dependent increase in inflammatory gene expression was attenuated by pretreatment with NF-κB inhibitors and antioxidant drugs (Fig. 5), suggesting that the effects of adipogenic G6PD overexpression are likely to be mediated through the oxidative stress and NF-κB signaling pathways.

Recent studies have revealed that the altered adipocyte biology in obesity enhances adipocyte-macrophage interactions, resulting in chronic inflammatory signals and insulin resistance (3,5). Because G6PD-overexpressing adipocytes change the expression of several adipocytokines, we hypothesized that the secretory factors of G6PD-overexpressing adipocytes might affect macrophage gene expression. In support of this idea, we observed that the expression of many macrophage-specific genes and the recruitment of THP-1 monocytes onto adipose tissue was remarkably increased in G6PD-overexpressing adipocytes (Figs. 6–8), implying that G6PD overexpression in adipocytes produces primary signals for inflamed adipocytes. Furthermore, our results provide an important clue to understand how macrophages and adipocytes communicate with each other and how, in obese subjects, peripheral macrophages respond to the signals from adipocytes with promoted infiltration into the adipose tissue.

In summary, we demonstrated a novel role of G6PD in obesity-induced metabolic disorders (Fig. 9). Variable environmental factors, including hyperglycemia, hyperinsulinemia, and the presence of cytokines, induce G6PD expression in adipose tissue. Subsequent elevations in G6PD in adipocytes increase oxidative stress; the activation of NF-κB signaling, which eventually stimulates inflammatory responses and insulin resistance; and dysregulation of adipocytokines. Increase of G6PD in adipocytes promotes macrophage recruitment and affects macrophage-specific gene expression, contributing to the chronic inflammation in obesity. Therefore, the identification of regulatory tools for G6PD activity may prove promising for treating metabolic disorders such as obesity-induced insulin resistance and chronic inflammation.

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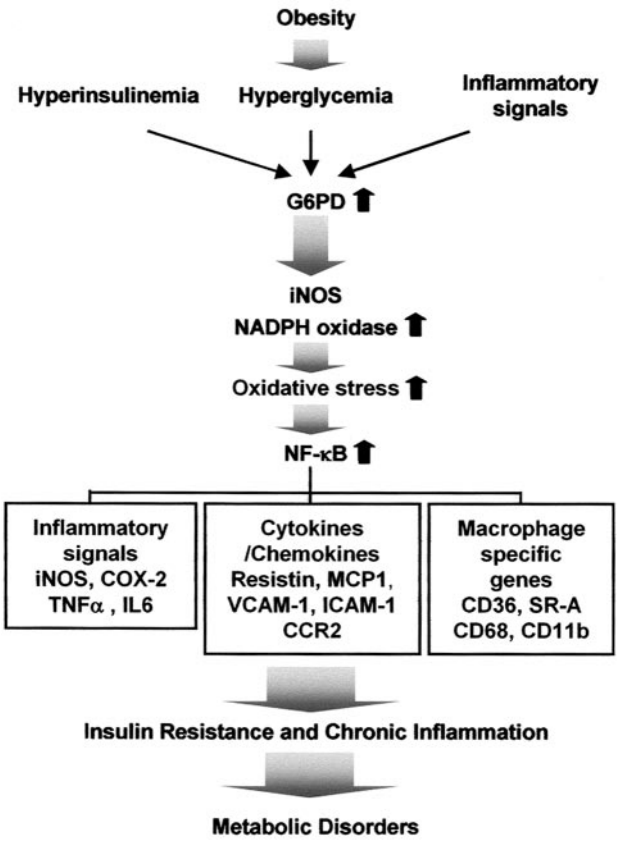


**FIG. 9. A model of G6PD-induced insulin resistance and chronic inflammation in adipocytes. Obesity-induced G6PD overexpression stimulates oxidative stress by increasing the expression of pro-oxidative enzymes, which are closely linked with NF-κB signals and their target gene expression, including cytokines, inflammatory signals, and macrophage-specific genes. These signals lead to metabolic disorders such as insulin resistance and chronic inflammation. The upward arrows indicate the increase of G6PD, iNOS, NADPH oxidase, oxidative stress, or NF-κB signaling. ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.**

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## REFERENCES

1. Zimmet P, Alberti KG, Shaw J: Global and societal implications of the diabetes epidemic. *Nature* 414:782–787, 2001
2. Kopelman PG: Obesity as a medical problem. *Nature* 404:635–643, 2000
3. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830, 2003
4. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ: Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 111: 932–939, 2005
5. Wellen KE, Hotamisligil GS: Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* 112:1785–1788, 2003
6. 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* 112:1796–1808, 2003
7. Hotamisligil GS: Inflammatory pathways and insulin action. *Int J Obes Relat Metab Disord* 27 (Suppl. 3):S53–S55, 2003
8. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I: Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 114:1752–1761, 2004
9. Dandona P, Aljada A, Bandyopadhyay A: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 25:4–7, 2004
10. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L: The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev* 17:189–212, 2001
11. Evans JL, Goldfine ID, Maddux BA, Grodsky GM: Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? *Diabetes* 52:1–8, 2003
12. Finkel T, Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. *Nature* 408:239–247, 2000
13. Huang H, Manton KG: The role of oxidative damage in mitochondria during aging: a review. *Front Biosci* 9:1100–1117, 2004
14. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404:787–790, 2000
15. Jenner P: Parkinson's disease, pesticides and mitochondrial dysfunction. *Trends Neurosci* 24:245–247, 2001
16. Aliev G, Seyidova D, Lamb BT, Obrenovich ME, Siedlak SL, Vinters HV, Friedland RP, LaManna JC, Smith MA, Perry G: Mitochondria and vascular lesions as a central target for the development of Alzheimer's disease and Alzheimer disease-like pathology in transgenic mice. *Neurol Res* 25:665–674, 2003
17. Yorek MA: The role of oxidative stress in diabetic vascular and neural disease. *Free Radic Res* 37:471–480, 2003
18. Schrauwen P, Hesselink MK: Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. *Diabetes* 53:1412–1417, 2004
19. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
20. Perreault M, Marette A: Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med* 7:1138–1143, 2001
21. Spolarics Z: Endotoxemia, pentose cycle, and the oxidant/antioxidant balance in the hepatic sinusoid. *J Leukoc Biol* 63:534–541, 1998
22. Spolarics Z: A carbohydrate-rich diet stimulates glucose-6-phosphate dehydrogenase expression in rat hepatic sinusoidal endothelial cells. *J Nutr* 129:105–108, 1999
23. Park J, Rho HK, Kim KH, Choe SS, Lee YS, Kim JB: Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance. *Mol Cell Biol* 25:5146–5157, 2005
24. Sadowski HB, Gilman MZ: Cell-free activation of a DNA-binding protein by epidermal growth factor. *Nature* 362:79–83, 1993
25. Tsai KJ, Hung IJ, Chow CK, Stern A, Chao SS, Chiu DT: Impaired production of nitric oxide, superoxide, and hydrogen peroxide in glucose 6-phosphate-dehydrogenase-deficient granulocytes. *FEBS Lett* 436:411–414, 1998
26. Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA: Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* 54:2305–2313, 2005
27. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE: Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293:1673–1677, 2001
28. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M: IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 11:191–198, 2005
29. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE: Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11:183–190, 2005
30. Rudich A, Kozlovsky N, Potashnik R, Bashan N: Oxidant stress reduces insulin responsiveness in 3T3–L1 adipocytes. *Am J Physiol* 272:E935–E940, 1997
31. Evans JL, Goldfine ID, Maddux BA, Grodsky GM: Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 23:599–622, 2002
32. Ogihara T, Asano T, Katagiri H, Sakoda H, Anai M, Shojima N, Ono H, Fujishiro M, Kushiyama A, Fukushima Y, Kikuchi M, Noguchi N, Aburatani H, Gotoh Y, Komuro I, Fujita T: Oxidative stress induces insulin resistance

by activating the nuclear factor-kappa B pathway and disrupting normal subcellular distribution of phosphatidylinositol 3-kinase. *Diabetologia* 47:794–805, 2004
33. Schwartz AG, Pashko LL: Dehydroepiandrosterone, glucose-6-phosphate dehydrogenase, and longevity. *Ageing Res Rev* 3:171–187, 2004
34. Kahn BB, Flier JS: Obesity and insulin resistance. *J Clin Invest* 106:473–481, 2000
35. Collino M, Aragno M, Mastrocola R, Gallicchio M, Rosa AC, Dianzani C, Danni O, Thiemermann C, Fantozzi R: Modulation of the oxidative stress and inflammatory response by PPAR-gamma agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion. *Eur J Pharmacol* 530:70–80, 2006
36. Da Ros R, Assaloni R, Ceriello A: The preventive anti-oxidant action of thiazolidinediones: a new therapeutic prospect in diabetes and insulin resistance. *Diabet Med* 21:1249–1252, 2004
37. Suganami T, Nishida J, Ogawa Y: A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. *Arterioscler Thromb Vasc Biol* 25:2062–2068, 2005
38. Moreno PR, Fuster V: New aspects in the pathogenesis of diabetic atherothrombosis. *J Am Coll Cardiol* 44:2293–2300, 2004
39. Da Ros R, Assaloni R, Ceriello A: Molecular targets of diabetic vascular complications and potential new drugs. *Curr Drug Targets* 6:503–509, 2005
40. Kim MS, Park JY, Namkoong C, Jang PG, Ryu JW, Song HS, Yun JY, Namgoong IS, Ha J, Park IS, Lee IK, Viollet B, Youn JH, Lee HK, Lee KU: Antiobesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat Med* 10:727–733, 2004
41. Ceriello A, Motz E: Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 24:816–823, 2004
42. Rosenson RS: Statins in atherosclerosis: lipid-lowering agents with anti-oxidant capabilities. *Atherosclerosis* 173:1–12, 2004
43. Cathcart MK: Regulation of superoxide anion production by NADPH oxidase in monocytes/macrophages: contributions to atherosclerosis. *Arterioscler Thromb Vasc Biol* 24:23–28, 2004
44. Zalba G, San Jose G, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, Diez J: Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. *Hypertension* 38:1395–1399, 2001
45. Carvalho-Filho MA, Ueno M, Hirabara SM, Seabra AB, Carvalheira JB, de Oliveira MG, Velloso LA, Curi R, Saad MJ: S-nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase b/akt: a novel mechanism of insulin resistance. *Diabetes* 54:959–967, 2005
46. Yasukawa T, Tokunaga E, Ota H, Sugita H, Martyn JA, Kaneki M: S-nitrosylation-dependent inactivation of Akt/protein kinase B in insulin resistance. *J Biol Chem* 280:7511–7518, 2005
47. Rolo AP, Palmeira CM: Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol Appl Pharmacol* 212:167–178, 2006
48. Leverve XM, Guigas B, Detaille D, Batandier C, Koceir EA, Chauvin C, Fontaine E, Wiernsperger NF: Mitochondrial metabolism and type-2 diabetes: a specific target of metformin. *Diabetes Metab* 29:6S88–6S94, 2003
49. Green K, Brand MD, Murphy MP: Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes (Review). *Diabetes* 53 (Suppl. 1):S110–S118, 2004
50. Ho E, Bray TM: Antioxidants, NFkappaB activation, and diabetogenesis. *Proc Soc Exp Biol Med* 222:205–213, 1999
51. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI: Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* 108:437–446, 2001
52. Mohamed AK, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP: The role of oxidative stress and NF-kappaB activation in late diabetic complications. *Biofactors* 10:157–167, 1999